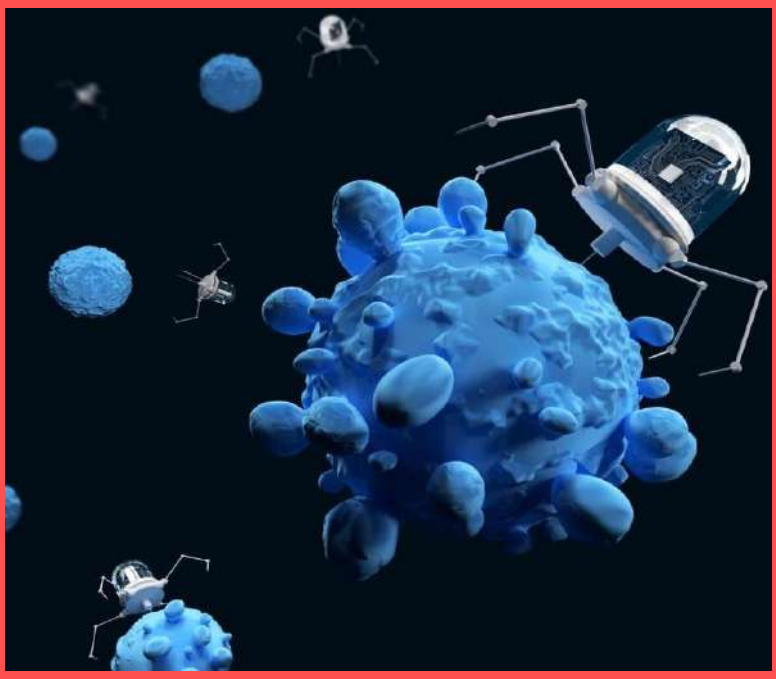
BIOCOMPATIBILITY AND SAFETY OF NANOPARTICLE-BASED DRUGS



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PREFACE

In recent years, nanoparticle-based therapeutics have demonstrated significant progress to overcome several major drawbacks of conventional drug formulations, such as poor solubility, rapid degradation, systemic toxicity, and limited bioavailability. These systems provide more precise drug delivery, improved pharmacokinetic profiles, and enhanced therapeutic efficacy. Advances in nanotechnology have enabled the fine-tuning of the physicochemical characteristics of nanoparticles, including size, morphology, surface charge, and surface functionalization, through advanced synthesis and characterization techniques. Due to the complex and often unpredictable interactions between nanoparticles and biological systems, ensuring biocompatibility, biodegradability, and long-term safety is of great importance. Therefore, a comprehensive preclinical evaluation encompassing both *in vitro* and *in vivo* studies is essential to evaluate toxicity, immunogenicity, and pharmacokinetic behavior. Although large-scale production, regulatory approval, and long-term safety evaluation still present challenges, nanoparticle-based drug delivery systems continue to hold great potential for advancing personalized and targeted medicine in the near future.

10/11/2025

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INTRODUCTION

In recent years, on the brink of an unprecedented global pandemic, nanoparticle-based vaccinations have played a crucial role in people's lives (Yang et al., 2024). In general terms, drugs are the active compounds in a pharmaceutical product (Soni et al., 2018). All medicines use pharmacologically active metabolites to treat disorders (Afzal et al., 2022). Nanoparticles are either organic or inorganic, small-scale matter in 10-100 nm, and they are produced with the help of nanotechnological tools, such as bottom-up and top-down synthesis methods (Khan et al., 2019). Nanoparticles can be used as therapeutics, and the best example of this is the nanoparticulate system developed for the global pandemic of COVID-19 that we have experienced in recent years (Schütz et al., 2013; Yang et al., 2024).

Nanoparticles can be classified into different types, such as carbon-based, metallic, polymeric, and lipid-based, and these are used in controlled drug delivery (Khan et al., 2019). Controlled drug delivery systems are more effective than conventional systems; therefore, nanoparticles have been used as a drug delivery system in recent years.

Conventional drugs can have disadvantages, mainly in passing the biological barriers (e.g., the blood-brain barrier (BBB)) and less selectivity. Nanoparticles have shown significant potential for overcoming biological barriers, as their ability to traverse such obstacles depends on precise characterization and optimization of their physicochemical properties (Wilczewska et al., 2012).

Biocompatibility is the ability of a biomaterial to perform its intended function in relation to medical therapy without causing any adverse local or systemic effects on the patient. The treatment offers significant benefits while simultaneously providing the optimal clinically relevant performance (Bruinink & Luginbuehl, 2011; Huzum et al., 2021). On the other hand, nanomaterials can cause adverse effects such as inflammation, toxicity, or immune triggering in biological systems (Yusuf et al., 2023); therefore, biocompatibility is crucial for nanoparticle-based drugs such as lipid nanoparticle-based vaccines produced for COVID-19. These vaccines improve mRNA transport into human cells while reducing immune system activation and toxicity (Mehta et al., 2023). Nanoparticles can be toxic or show toxic effects such as undesired immune responses, mitochondrial dysfunction, protein denaturation, or alterations in the cell cycle (Liu et al., 2020). Furthermore, they can accumulate in tissues and organs (Nishimori et al., 2009). Several factors can influence the toxicity of nanoparticles, including the dose, chemical structure, and size (Recordati et al., 2016). Therefore, safety tests are crucial for developing and approving nanoparticle-based drugs. The Food and Drug Administration (FDA) evaluates and determines the safety of pharmaceutical compounds.

These processes include pre-clinical and clinical studies (FDA | 2024, Nov 18). Model organisms and cell culture techniques (e.g., zebrafish models and 3D cell culture) are used for pre-clinical studies, such as (Pensado-López et al., 2021; Wang et al., 2021). Lipid nanoparticle-based mRNA vaccines can be an example for clinical trials. A sequential process consisting of distinct phases, each designed to systematically evaluate specific targets and objectives (Hou et al., 2021).

1. Drugs

1.1. Medicine and Drugs

Human civilizations have had a strong connection with their surroundings since ancient times, and they have utilized many components to produce food and medicine (Jamshidi-Kia et al., 2017). Drugs are specialized products that are used to cure diseases or improve health (Lan et al., 2025). Natural products are the basis of traditional medicine. They contain a variety of chemical components that determine their pharmacological behaviors. However, even the most effective substances are relatively ineffective when taken alone (Kimura et al., 2000). Drug combination therapies, which may provide increased efficacy through the synergistic effects of combined therapies, are gaining popularity. Cell therapy can produce better results when combined with other cancer treatments, including radiation and chemotherapy (Bagley & Wages, 2025). Liu *et al.* used oxidized hyaluronic acid loaded with the chemotherapeutic drug daunorubicin and the photosensitizer methyl aminolevulinate as a nanocarrier for the treatment of breast cancer. They developed a pH-sensitive, self-

assembling drug delivery system, enabling synchronized drug release and combination therapy (Liu et al., 2025).

In recent years, numerous nanotechnological methods have been developed for use in medicine. Utilizing physicochemical, biological, and engineering concepts to produce nanoscale structures, nanomedicine has expanded (Henn et al., 2024). Nanomedicine is a new field that aims to change drug delivery systems and clinical practice by taking advantage of the unique properties of nanoparticles. Nanomedicine focuses on developing highly efficient drug delivery systems using nanoparticles (Singh et al., 2024). Bharwaj *et al.* used a drug delivery system using chitosan-coated lipid-polymer hybrid nanoparticles to enhance the solubility, bioavailability, and controlled release of erucine, a lipophilic compound. They reported that these nanoparticles used in this system functioned as a controlled nanocarrier system, offering solutions to pharmaceutical challenges such as solubility, bioavailability, and controlled release (Bhardwaj et al., 2025). Feng *et al.* tested polyethylene glycol (PEG)-Poly(lactic-co-glycolide (PLGA) nanoparticles for the treatment of inflammatory intestinal disease. These nanoparticles were loaded with adalimumab, which is an antibody, and tested for oral delivery. Adalimumab-loaded PEG-PLGA nanoparticles showed higher cellular uptake and intestinal epithelial permeability under inflammatory conditions (Feng et al., 2025).

1.2. Categorization of Drugs

In general terms, drugs can be classified into six main categories. These are oncologic (anticancer), antidiabetic, central nervous system, cardiovascular, anti-infective, and gastrointestinal drugs (Zhao et al., 2025). Anticancer drugs primarily affect tumor cells, tumor vasculature, the immune system, or the endocrine system. The mechanisms of anticancer drugs include causing DNA damage, blocking cell cycle progression, and inhibiting signaling pathways. Their usage varies depending on the type and stage of the disease and the target area of the drug. Some new agents with new mechanisms of action are drugs that are difficult to classify into the groups of classic chemotherapy, hormones, tyrosine kinase inhibitors, and monoclonal antibodies (Ostios-Garcia et al., 2024). Hinojosa *et al.* used an aerobic granular sludge system in a continuous-flow reactor; they investigated the effects of three common chemotherapy drugs (cyclophosphamide, tamoxifen, and methotrexate) at different doses on physicochemical parameters and drug removal efficiency. As a result of the evaluations, it has been shown that at medium and high concentrations, pharmaceuticals cause adverse effects on physicochemical parameters. Hence, these pharmaceuticals can seriously alter treatment performance (Castellano-Hinojosa et al., 2025). Yadav *et al*. synthesized pyrimidine-based molecules combined with triazole or imidazole to produce potent anticancer drugs. The obtained compounds were evaluated for anticancer activity. These compounds were also found to activate the secondary cell death mechanism through apoptosis in cancer cells. They

reduced oxidative stress, induced apoptosis, and stopped the cell cycle in the G2/M phase (Yadav et al., 2025).

Antidiabetic drugs affect different biological targets to lower blood glucose levels. The most common mechanisms of action include increasing insulin secretion, reducing glucose production and absorption, or increasing excretion. For example, sulfonylurea derivatives increase insulin secretion by closing KATP channels found in β-cells. Metformin, on the other hand, increases insulin sensitivity and reduces glucose absorption by suppressing gluconeogenesis in the liver (Pandey et al., 2025). Kondalkar *et al.* investigated the herb-herb interactions and herb-drug interactions of Madhukiran formulations in the streptozotocin-nicotinamide-induced rat model. In addition, antidiabetic, cardioprotective, hepatoprotective, pancreatoprotective, and nephroprotective effects were investigated. As a result, Madhukiran formulations had significant antidiabetic activity in rats (Kondalkar et al., 2025). Bansal et al. synthesized vanadium pentoxide nanoparticles using chitosan and its derivatives to enhance antioxidant and antidiabetic activity. Among the synthesized nanoparticles, vanadium pentoxide nanoparticles decorated with chitosan-salicylaldehyde showed the highest antidiabetic activity (Bansal et al., 2025).

Central nervous system (CNS) drugs are developed using more specialized mechanisms than drugs targeting other organs due to the complex structure of the brain and the protective effect of the BBB. The mechanisms of action of CNS drugs are generally based on the regulation of gene expression. Prominent drugs in this context are antisense oligonucleotides (ASOs) and short interfering RNAs

(siRNAs). The most significant challenge of CNS drugs is crossing the BBB; therefore, drugs are usually administered via intratecal (IT) or intracerebroventricular (ICV) injections, which are routes of direct administration to the brain (McCartan et al., 2023). Jadhav *et al.* aimed to improve the efficacy of anti-tuberculosis drugs in the treatment of central nervous system tuberculosis by intranasal administration of methyl-β-cyclodextrin microparticles. Intranasal insufflation of anti-tuberculosis microparticles administered for four weeks resulted in a significant reduction in mycobacterial load in the brain compared to the untreated group (Jadhav et al., 2025).

Cardiovascular drugs are medications used in the prevention and treatment of heart and vascular diseases, each with different mechanisms of action. They are commonly used in conditions such as heart failure, atherosclerosis, hypertension, arrhythmia, and vascular occlusion. For example, colchicine binds to β-tubulin and reduces neutrophil activation by inhibiting microtubule polymerization. Sotagliflozin functions as a dual inhibitor of SGLT1 and SGLT2. This inhibits glucose absorption in the intestines and glucose reabsorption in the kidneys. Thus, it lowers blood sugar levels and protects heart function by reducing circulatory load (Tamargo et al., 2024). Medina et al. reported the challenge of identifying key therapeutic targets and drugs for personalized cardiology. To address this, extensive *in silico* analyses, such as single-stranded RNA sequencing data, functionalization analyses, and protein interaction networks, were conducted. The targets of the 15 drugs were included in a protein-protein interaction network created after comparison with the drug-

target database. The most promising drug/target combinations were then identified using network proximity and cell type expression data. The importance of testing these in future in vitro and in vivo experimental validations was highlighted. As a result, 15 effective drugs have been identified in clinical trials for late-stage cardiovascular disorders (Medina et al., 2024).

Anti-infective drugs generally refer to substances used against infectious agents. Antibiotics are the most widely and comprehensively used group among these. Antibiotics are chemical substances that inhibit or eliminate bacterial growth. Antibiotics have four main mechanisms of action, inhibition of DNA replication, protein synthesis, cell wall synthesis, and folic acid metabolism. Examples of anti-infective drugs include Fluoroquinolones, Aminoglycosides, Beta-lactams, and Sulfonamides (Halawa et al., 2023). Zheng *et al.* suggested a new surface-enhanced Raman scattering method for detecting anti-infective drugs in surrounding waters that was based on the vortex aggregation of Ag nanoparticles. The quantitative relationships obtained by this method were successful in terms of accuracy and stability. This method was also reported as an easy and reproducible method (Zheng et al., 2022).

Gastrointestinal drugs are therapeutic agents that are usually administered orally or rectally and exert their effects by being absorbed through the gastrointestinal system. Due to the length of the gastrointestinal tract, varying pH levels, and mucosal barriers, these drugs may experience bioavailability loss, making their formulation a key determinant of drug efficacy. In addition to drugs such as

sitagliptin, dapagliflozin, and meloxicam, which are administered via the gastrointestinal route for systemic effects, there are also drugs such as berberine, curcumin, and silymarin that have a local effect and are used directly in gastrointestinal diseases. The general mechanism of these drugs is to increase gastrointestinal absorption, deliver the drug more easily to the target site, and suppress local inflammation, especially in conditions such as inflammatory bowel disease (Chen et al., 2025). Villela *et al.* investigated particles with a core/shell PEG structure to enable the use of oleic acid as an effective gastrointestinal drug delivery system. The results showed that the composition of the liquid medium significantly affects particle stability and degradation behavior. It highlights the functional potential of such core/shell structures for drug delivery applications in the gastrointestinal tract (Villela et al., 2024).

2. Nanoparticles

2.1. Nanoparticle Synthesis, Design, Characterization, and Applications

Nanoparticle synthesis methods can be mainly divided into top-down and bottom-up approaches. In general terms, the top-down synthesis approach uses the bulk material to synthesize nanoparticles. Different types of methods can be defined under top-down approaches, such as sonication (Ruiz et al., 2022a), laser ablation (Rashid et al., 2021), ball milling (Hernández-Varela et al., 2021), lithography (Desponds et al., 2021), sputtering (Barman & Sarma, 2020), etching (Sempel et al., 2024), pulse wire discharge (Gao et al., 2020), electric

explosion (Glazkova et al., 2022a), electrospinning (Figure 1) (Huan et al., 2022).

Sonication is a method that uses sound energy to agitate the nanoparticles in the suspension. Sonication enables the breakup and dispersion of smaller droplets in the phase. Therefore, when the power of sonication increases, there is a reduction in particle size due to increased energy transfer. To avoid temperature rise, systems with multiple sonication cycles were also used with 10-second pauses between sonication steps (Mahbubul et al., 2016; Ruiz et al., 2022b). Laser ablation refers to removing atoms or tiny portions of material using a focused pulsed laser beam or a continuous wave. Laser ablation in liquid can stimulate chemical reactions during laser irradiation, and this property can be used to control particle size and functionalize the surface of nanoparticles to prevent deposition and precipitation. It is recognized as a relatively fast, easy-to-manipulate, and cost-effective technique (Khashan et al., 2021). Ball milling is a widely used approach to produce nanoparticles, which involves mechanically reducing bulk materials to the nanoscale. This process requires the control and optimization of the physicochemical parameters involved in the different stages of this process (Benchelia et al., 2024; Hernández-Varela et al., 2021). Lithography selectively exposes and transfers patterns to a surface using masks, light, or electron beams. There are different types of lithography, such as photolithography, interference lithography, electron beam lithography, mold-based lithography (nanoimprint and soft lithography), nanostencil lithography, and nanosphere lithography (McGrath et al., 2021; Zheng et al., 2024).

Sputtering is a method that removes nanoscale particles from a target, the bulk material, using bombardment. There are different types of sputtering methods, such as magnetron, neutronic, and ion beam sputtering (Chiovaro et al., 2025; Lee et al., 2025; Lu et al., 2025; Shulga, 2024). Etching is the process that involves creating nanoparticles and using chemicals to remove nanolayers from wafer surfaces or portions of the material to achieve desired structural, functional, or aesthetic properties (Ayvazyan & Jacoby, 2023; Zhang et al., 2023). The pulse wire discharge technique generates nanoparticles by cooling the metal wire vapor created by pulsing current with an ambient gas. The process includes vaporization, plasma production, and condensation, with particle size and characteristics determined by energy input and surrounding circumstances (Lee et al., 2023). An electric explosion occurs when a thin metal wire is subjected to a strong current pulse, resulting in explosion, evaporation, and ionization (Glazkova et al., 2022b; Lozhkomoev et al., 2023). Electrospinning is a technique that uses a high electric field on a polymer solution for large-scale nanofiber and nanoparticle production (Huan et al., 2022).

Besides the top-down approach, various bottom-up approaches exist, such as thermal decomposition (Tomar & Jeevanandam, 2020), hydrothermal synthesis (Mohan et al., 2020), sol-gel method (Patel et al., 2022), and green synthesis (Figure 1) (Aldeen et al., 2022). According to these examples, the top-down approach uses atomic or molecular species to synthesize nanoparticles. Numerous methods within this approach involve the synthesis of nanoparticles through

chemical interactions, utilizing the inherent properties of the constituent chemicals.

The thermal decomposition method uses heat energy to break chemical bonds, and this break causes material fragmentation. When the precursor material is heated, it undergoes a chemical transition, which usually releases gases and produces smaller particles (Tomar & Jeevanandam, 2022; Zeng et al., 2023). Hydrothermal synthesis generates nanoparticles by chemically reacting to form nanomaterials at the proper temperature and pressure. This procedure usually entails dissolving or suspending a precursor material in water, which functions as a solvent (Farghadin et al., 2023; Guo et al., 2023). In the sol-gel method, the sol (solution) progressively transforms into a gel-like network with a liquid and solid phase. The sol thickens, and the liquid phase becomes trapped within the expanding solid network, yielding a gel with tiny nanoparticles suspended in the liquid matrix (Amor et al., 2022). Green synthesis uses plant extracts instead of industrial chemicals to generate nanoparticles, which indicates that this process can be defined as eco-friendly. Plant extracts include bioactive chemicals such as polyphenols, flavonoids, and terpenoids, which reduce and stabilize agents during nanoparticle production (Pushpamalini et al., 2021; Rajendrachari et al., 2021).

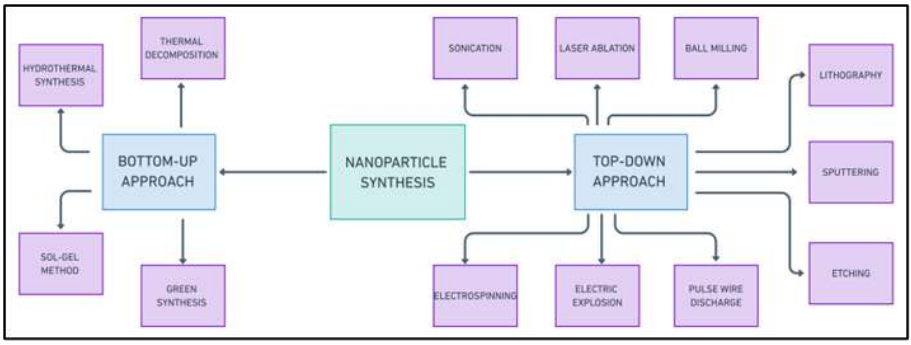

Figure 1: Summary of nanoparticle synthesis methods.

Nanoparticle design and synthesis are crucial in many aspects, such as safety, biocompatibility, and bioavailability. It depends on the intended use, which determines adjustable factors such as material selection, size, shape, surface properties, loading efficiency, biodegradability, biocompatibility, and toxicity. Biopolymers are frequently chosen for material selection due to their high biocompatibility. Surface properties affect the functionality of the nanoparticles, such as in the delivery of nanoparticle-based drugs, where the receptor binding site and charge are crucial for cellular uptake. Therefore, comprehensive characterization is imperative in the nanoparticle design process to validate and optimize their physicochemical properties, functional performance, and biological interactions (Desai et al., 2024; Nie et al., 2024; Ortiz-Perez et al., 2024; Stenspil & Laursen, 2024; Wang et al., 2024; Zhang et al., 2024).

Various techniques are used depending on the type of nanoparticle and property being analyzed to accurately determine their physicochemical, structural, and functional characteristics (Khan et al.,

2022). Physical characterization techniques provide insights into size, shape, and structural features. For instance, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) enable high-resolution imaging for particle morphology and size distribution (Singh et al., 2021; Wilson et al., 2021). Dynamic Light Scattering (DLS) measures hydrodynamic size in colloidal systems, while Atomic Force Microscopy (AFM) provides three-dimensional surface topography (Zhang et al., 2024). Crystalline structure and particle size are typically analyzed using X-ray Diffraction (XRD). Determining the oxidation states, functional groups, and elemental composition is the main goal of chemical characterization (Endla, 2022). X-ray Photoelectron Spectroscopy (XPS) and Energy-Dispersive X-ray Spectroscopy (EDS) are used to determine the elemental and chemical states (Kumar et al., 2022; Li et al., 2021). Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy are techniques for characterizing surface functional groups and molecular interactions (Qayyum et al., 2025; Vankudoth et al., 2022). Zeta potential analysis, which measures surface charge in colloidal systems, is used to assess surface characteristics that are crucial for stability and interactions (Gaur et al., 2023). Contact Angle Measurements assess wettability and surface hydrophilicity or hydrophobicity, which is essential for understanding nanoparticle behavior in various environments (Rathnaraj et al., 2022). Optical techniques examine how light interacts with nanoparticles (Gaur et al., 2023). While absorbance and band gap characteristics are determined using UV-visible spectroscopy, luminescent nanoparticles are measured using fluorescence and

photoluminescence (PL) spectroscopy (Ray et al., 2015; Vankudoth et al., 2022). Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) are used to evaluate thermal stability and phase transitions (Miao et al., 2018; Rami et al., 2021). Magnetic nanoparticles are evaluated for their magnetic characteristics using Vibrating Sample Magnetometry (VSM) and Superconducting Quantum Interference Devices (SQUID), which enable measurements of magnetization and coercivity (Mosleh et al., 2025). Biological characterization investigates the interactions between nanoparticles and biological systems, focusing on biocompatibility and toxicity, such as protein corona studies analyzing biomolecule adsorption that affects cellular uptake (Maillard et al., 2025; Juling et al., 2017). Small-Angle Neutron Scattering (SANS) provides insights into the size, morphology, and internal organization of nanoparticles by exploiting neutron scattering at very small angles, enabling nanoscale structural characterization (Hilburg et al., 2025; Yokaichiya et al., 2018). Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) allows precise determination of particle size distribution through detection and quantification of the elemental composition of individual nanoparticles (Pusuwan & Siripinyanond, 2024). Ellipsometry is employed to probe the optical characteristics of nanoparticles by monitoring variations in the polarization state of reflected light (Mandal et al., 2023; Zhang et al., 2022). Capillary Electrophoresis (CE) enables nanoparticle separation based on their charge-to-size ratio in an applied electric field, thereby facilitating accurate size and mobility analysis (Adelantado et al., 2024; Siebert et al., 2023). Small-Angle X-ray

Scattering (SAXS) is extensively used to elucidate structural parameters, particle size distribution, and shape by examining the angular dependence of X-ray scattering at low angles (Schlattmann et al., 2024; Yokaichiya et al., 2018). Resonance Light Scattering (RLS) provides a sensitive approach for assessing nanoparticle aggregation and size distribution, as resonance and aggregation phenomena significantly enhance scattering signals (Gong et al., 2020; Xi et al., 2025).

Nanoparticles exhibit a comprehensive range of applications across various fields (Figure 2) such as electronics (Peng et al., 2024), agriculture (Khattiya et al., 2025), food industry (Babaei et al., 2025), automotive industry (Güdümcüoğlu et al., 2025), cosmetics (Sallustio et al., 2024), and medicine (Ghasemi et al., 2025). Nanoparticles play a crucial role in the medical field, particularly in drug delivery (Sadigh et al., 2025), diagnostics (Espinola-Portilla et al., 2025), and biopharmaceutical applications (Chen et al., 2025) such as Au nanoparticles used for the development of biosensors (Ahmad et al., 2021), graphene-oxide used to enhance stem cell proliferation (López-García et al., 2024), poly(lactic-co-glycolic) acid (PLGA) nanoparticles are used for controlled drug release (Kelle et al., 2025a), lipid nanoparticles (LNP) used as drug carriers for the delivery of mRNA corresponding to COVID-19 vaccines (Sahin et al., 2020).

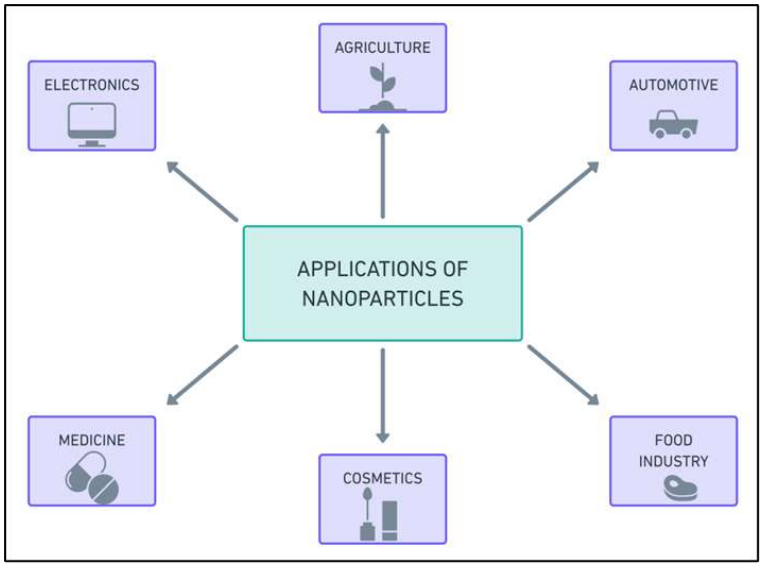

Figure 2: Summary of applications of nanoparticles.

2.1.1. Metal-Based Nanoparticles

Metal-based nanoparticles (MNPs) can be broadly divided into metal and metal oxide nanoparticles (MONPs) (Lee et al., 2025). Different types of metals can be used for the synthesis of MNPs, such as Silver (Ag) (Ravimoorthy et al., 2025), Mercury (Hg) (Tyszczuk-Rotko et al., 2016), Chromium (Cr) (Geetha et al., 2016), Iron (Fe) (Harshiny et al., 2015), Gold (Au) (Syed et al., 2016) (Figure 3), and Zinc (Zn) (Osuntokun & Ajibade, 2016) Besides these metals, MOs are also involved in nanoparticle synthesis, such as, zinc oxide (Bajpai et al., 2016), and cerium oxide (Kim & Chung, 2016).

MNPs cause toxicity primarily by releasing metal ions and producing reactive oxygen species (ROS). These reactions can damage DNA, proteins, and lipids, resulting in inflammation, apoptosis, or

necrosis. Enhancing the biocompatibility and stability of MNPs can be achieved by coating them with polymers such as chitosan. Since most metals carry a positive surface charge, these polymers are typically negatively charged. The electrostatic difference between the two promotes self-assembly and stable integration (Figure 3) (Amor et al., 2024).

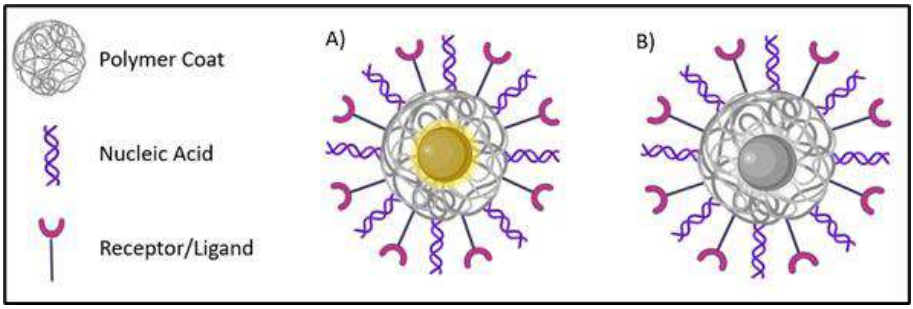

Figure 3: Illustration of (A) polymer-coated Au-nanoparticle-based drug and (B) polymer-coated Ag-nanoparticle-based drug (Created with BioRender.com).

MNPs are an effective catalyst due to their high electrical conductivity, electrocatalytic ability, and many electrically active sites. Nevertheless, the MNPs lack dedicated channels for the transport of reactants and products. The size of MNPs decreases with the decrease in thermodynamic stability. Small MNPs contribute to aggregation into large structures during catalysis due to their high surface energy. To increase the stability of MNPs and prevent aggregation, these particles are generally not uniform and stabilized using a carrier (Li et al., 2024). Because of their potential to improve chemical and thermal stability

when combined with hydrophobic or hydrophilic drugs or macromolecules, MNPs have attracted interest as an antifungal agent. Although Ag nanoparticles have been employed extensively for their antibacterial qualities, research is now focusing on alternative MNPs due to the high cost and possible toxicity of Ag-based materials to humans (Yadav et al., 2025). MNPs have been used to evaluate their antimicrobial activity against specific pathogens. Malakar *et al*. reported on the synthesis of Zinc oxide particles by coating with Sophorolipid and characterized them via various techniques such as FTIR, XRD, TGA, and AFM (Malakar et al., 2025). In another study, the anticancer potential of MNPs against a HepG2 (liver cancer) cell line was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Three different nanoparticles (AgO_2, CeO_2, CuO_2) were synthesized using *Trianthima Portulacastrum* and *Chinopodium Quinoa* leaf extracts. The synthesized MNPs were characterized via various techniques such as SEM and XRD. The results indicated that the green-synthesized nanoparticles had unique crystalline structures with average diameters between 8 and 24 nm. HepG2 cell viability was shown to decrease in a concentration-dependent way via *in vitro* cytotoxicity tests, with CeO_2 and CuO_2 nanoparticles showing the strongest anticancer activity and a low level of toxicity to normal HEK-293 (human embryonic kidney) cells (Younas et al., 2021).

2.1.2. Carbon-Based Nanoparticles

Materials that include carbon as their primary structural component are referred to as carbon-based materials. High-performance carbon materials are widely used in scientific research and commercial applications due to their remarkable physicochemical characteristics (Zhang & Yan, 2025). Carbon-based nanoparticles (CNPs) can be broadly divided into fullerene (Figure 4A) (Talaei et al., 2025), carbon nanotubes (CNTs) (Figure 4B) (Soyalp et al., 2025), nanodiamonds (Figure 4C) (Li et al., 2025), graphene, and graphene oxide (Figure 4D) (Patel et al., 2025).

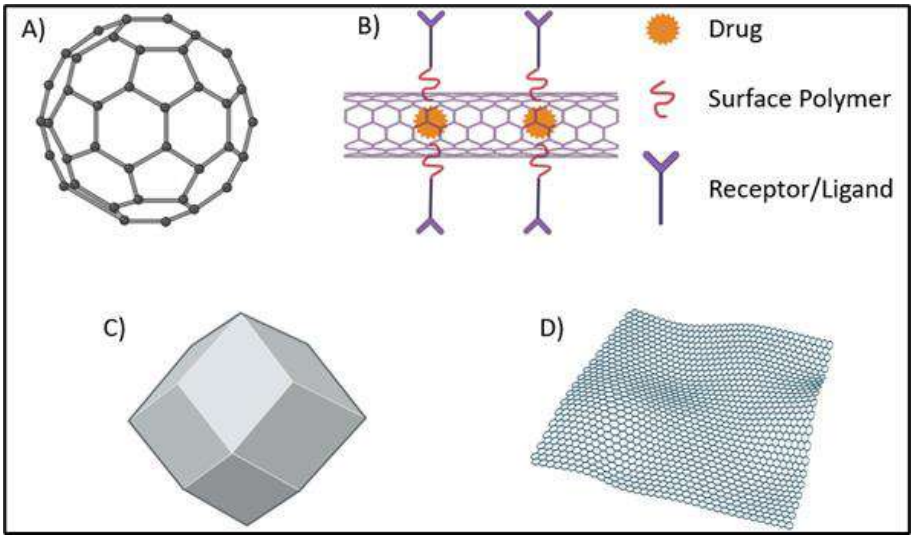

Figure 4: Illustration of (A) fullerene, (B) polymer-coated carbon nanotube-based drug, (C) nanodiamond, (D) graphene (Created with BioRender.com).

Fullerene (C60) and its functionalized derivatives were investigated as drug delivery nanocarriers. To evaluate the synthesized material's size, shape, and chemical interactions, TEM, FTIR, and UV-Vis simulations were used. The targeted administration of drugs, including doxorubicin, paclitaxel, and berberine, was made possible by the high drug-loading capacity, high biocompatibility, and tunable surface functionality of these fullerene-based systems. Several formulations showed significant anticancer activity and pH-responsive release *in vitro* (Borisenkova et al., 2024).

An oriented nanofiber scaffold composed of poly(ε-caprolactone) (PCL), gelatin, and CNTs was produced using the electrospinning technique. The incorporation of CNTs endowed the scaffold with elastic properties comparable to native wound tissue. The morphological characteristics of the synthesized scaffold were characterized via SEM. The *in vitro* experiments demonstrated that the CNT-enriched scaffold significantly enhanced wound healing. Quantitative analysis revealed that the wound closure rate achieved with the synthesized material was 91.86%, in contrast to 66.75% observed in the control group, indicating a substantial improvement in wound healing efficacy (Chen et al., 2025). In another study, green-synthesized polyaniline/graphene oxide nanosheets were used for peripheral nerve regeneration. Synthesized material was characterized via DLS, FTIR, SEM, and TEM techniques. This material exhibited enhanced electrical conductivity (~0.3 S/cm), high porosity (32–42%), and favorable biodegradability. Mechanical testing revealed a tensile strength of approximately 31 MPa, while *in vitro* biocompatibility assays demonstrated higher than 90% Schwann

cell viability. These properties underscore the potential of the synthesized material as a biocompatible, conductive, and mechanically robust multichannel nerve conduit for peripheral nerve regeneration (Zaman et al., 2024).

2.1.3. Polymer-Based Nanoparticles

Polymer-based nanoparticles are more cost-effective and easier to synthesize compared to other nanoparticles that are used in drug delivery systems (Ma et al., 2025). The ratio of hydrophilic to hydrophobic chains can be adjusted to produce nanoparticles with different characteristics. Biodegradable polymers like PLGA, PCL, and polylactide can be employed as hydrophobic chains, while biocompatible PEG is typically used as a hydrophilic chain (Iioka et al., 2025). Polymer-based nanoparticles represent a convenient nanoparticle platform for the delivery of both water-soluble and lipid-soluble compounds. Polymer-based nanoparticles can provide various functionalities such as drug release profiles, biodegradability, stability in biological applications, and adjustable particle sizes (Figure 5) (Dave et al., 2025a).

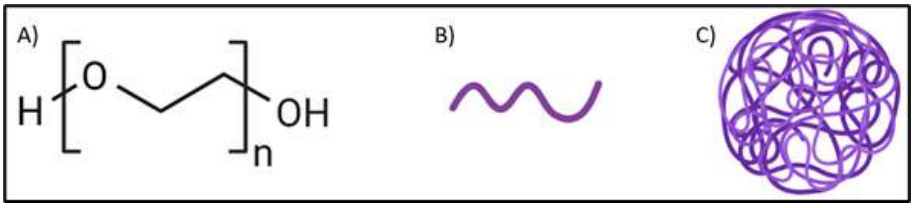

Figure 5: (A) Chemical structure of PEG, (B) illustration of PEG, and (C) polymer-based nanoparticle (Created with BioRender.com).

The potential of drug-loaded polymer nanoparticles to treat various diseases is being extensively studied. These nanoparticles are characterized using techniques such as DLS and TEM. They are produced by combining pre-made degradable polymers with drugs through emulsification methods like emulsification-solvent evaporation or nanoprecipitation. A major drawback of this production method is the creation of low-concentration nanoparticles with low drug loadings and rapid, early drug release upon administration. This reduces therapeutic effectiveness and can cause toxic effects. In a study, polymer nanoparticles were conjugated with N-acetyl cysteine and used for treating acute lung injury. Their application showed remarkable success for improving pulmonary edema, decreasing inflammatory cell presence, and lowering pro-inflammatory cytokine levels. Therefore, they demonstrated a significant ability to lessen harmful effects on lung tissue and provided effective protection against inflammation (Muhammad et al., 2025).

2.1.4. Lipid-Based Nanoparticles

Research conducted in 1964 supported the significance of lipids as permeability barriers in biological membranes. They demonstrated that ovolecithin (egg yolk phosphatidylcholine) dispersions in aqueous media spontaneously formed liposomes. Liposomes are spherical vesicles having an aqueous core surrounded by phospholipid bilayers. The drug molecules are encapsulated within the central aqueous core. Liposomes can carry both hydrophilic and lipophilic drugs. They are commonly used in pharmaceutical applications to deliver drugs in a

targeted and controlled drug delivery (Figure 6A) (Bangham, Standish, & Miller, 1965; Bangham, Standish, & Watkins, 1965; Bangham & Horne, 1964). Nevertheless, their stability is challenged by lipid oxidation, aggregation, and environmental sensitivity, limiting their practical applications (Zhao et al., 2025).

The production of multifunctional LNP functionalized with CP-2 peptide (CP-2-LNPs) was proposed for the detection and treatment of Alzheimer's disease (AD) earlier. Synthesized LNPs are characterized via TEM and DLS. By selectively binding to toxic amyloid-β oligomers (AβOs), CP-2-LNPs prevent aggregation and reduce the neurotoxicity caused by Aβ. It was demonstrated that CP-2-LNPs successfully passed through the BBB transgenic mouse models and were evaluated as a potential biomarker for early AD detection (Senapati et al., 2024). Another study demonstrates the potential of anionic LNPs, which lack adjuvants and antigens. The produced LNPs promote dendritic cell MyD88 signaling pathway by activating T cells and enhancing the immunological response. Synthesized LNPs are characterized via TEM and DLS. Due to their ability to cross the BBB and accumulate in lymph nodes, these LNPs enhance adaptive immunity by increasing Th1 and $CD8^+$ T cells. They have been shown to inhibit tumor growth and improve survival when administered with an antigen in the MC38 (colon cancer mouse model). These findings suggest that LNPs with a strong negative charge could be considered safe and effective nanosystems for immunotherapy (Guo et al., 2024).

Solid lipid nanoparticles (SLNs) (Figure 6B) can be used in a wide range of biological applications since they are typically spherical

in shape with a solid lipid core, and sizes between 50 and 1000 nm. Moreover, solid lipids stabilized by surfactants form SLNs. At body and room temperatures, the lipid core stays completely solid. By stabilizing the particle's surface, the surfactant molecules keep it from aggregating. It is possible to carry out drugs in this solid lipid matrix. The crystalline form of the solid lipid may limit the drug-loading capacity of SLNs, which combine the benefits of conventional lipid carriers with enhanced stability and controlled drug release (Alotaibi et al., 2025). Lauric acid and tea tree oil were combined to produce SLNs, and their physicochemical characteristics and antibacterial capabilities were examined. According to characterization analyses, the SLNs were uniformly dispersed in a spherical structure, ranged in size from 239.58 to 344.7 nm, and had a zeta potential between -17.8 and -13.1 mV. SLNs at a dosage of 12.5 mg/mL demonstrated an antibacterial activity and inhibited the growth of *Pseudomonas aeruginosa* by inducing damage to the cell membrane (Motsoene et al., 2025). Another study evaluates the efficiency of green SLNs, which are produced in a low-energy and solvent-free way, as carriers of cosmetic ingredients. The stability and skin permeability of SLNs produced from mangoes and shea that were loaded with UV filters and anti-aging compounds were investigated. Synthesized SLNs are characterized via TEM, SEM, and DLS. It demonstrated that SLNs enhanced the distribution of compounds to the dermis. The potential of SLNs as an eco-friendly and efficient cosmetic carrier system was demonstrated by the SLN-containing serum's success in enhancing human skin hydration and elasticity, as well as its physicochemical stability (Bozza et al., 2025).

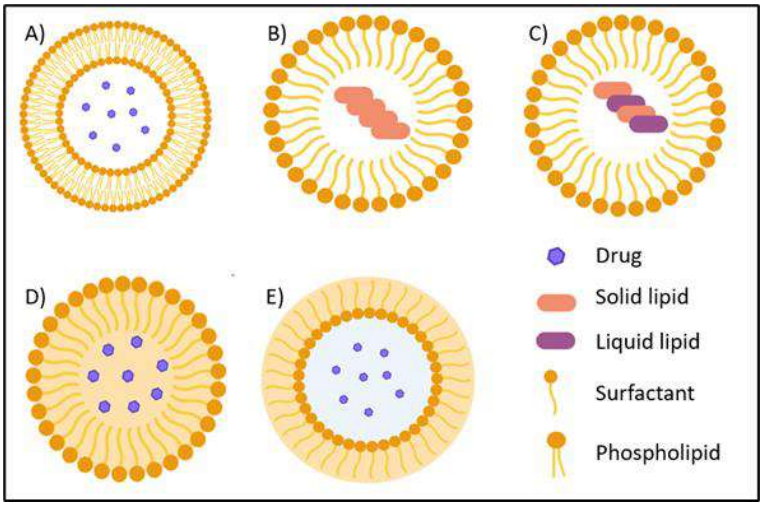

Figure 6: Illustration of (A) liposome, (B) solid lipid nanoparticle, (C) nanostructured lipid carriers, (D) water/oil nanoemulsion, (E) oil/water nanoemulsion (Created with BioRender.com).

Nanostructured lipid carriers (NLCs) are designed to overcome the limitations of SLNs. They are composed of a mixture of solid and liquid lipids (Figure 6C), resulting in an inconsistent crystal structure. This structural irregularity allows for more space within the lipid matrix, increasing drug loading capacity. The mixed lipid phase also allows for greater control over drug release profiles, making NLCs more flexible than SLNs in drug delivery (Carvalho et al., 2024). The efficacy of chitosan-coated NLCs in intranasal drug administration for improving the brain bioavailability of synaptic acid in the treatment of AD was investigated. Chitosan-coated synaptic acid-loaded NLCs have an ideal particle size of less than 200 nm, allowing for prolonged retention in the nasal mucosa and enhanced brain targeting. Synthesized

NCLs are characterized via XRD, FTIR, and DLS. The *in vivo* pharmacokinetic studies indicated that chitosan-coated synaptic acid-loaded NLCs highlighted synaptic acid exposure in brain tissue by 2.6-fold while also increasing the elimination half-life by 1.7-fold. Furthermore, histological studies proved biocompatibility, with controlled drug release leading to significant reductions in oxidative stress and neuroinflammation. The findings indicated that the chitosan-coated NLC system is an effective approach for the treatment of AD by intranasal drug delivery (Prabakaran et al., 2025). Transdermal administration of hesperidin-loaded NLCs using soluble microneedles was researched. Synthesized NCLs are characterized via SEM, TEM, FTIR, and XRD. The *in vivo* pharmacokinetic studies of the hyaluronic acid-based microneedle form supported controlled and targeted drug release by improving Hesperidin bioavailability and prolonging the elimination half-life. Histological studies indicated that the system has anti-obesity efficiency by reducing adipogenesis and inducing browning of white adipose tissue (Gopan et al., 2025).

Nanoemulsion (NE) (Figure 6D and E) is a colloidal dispersion that is kinetically stable, although thermodynamically unstable, with droplets ranging in diameter from 20 to 200 nm (Davis et al., 1987). Oil-in-water (O/W) systems (Figure 6D) have oil droplets with lipophilic drugs dispersed in a continuous aqueous phase, while water-in-oil (W/O) (Figure 6E) systems have small aqueous droplets with hydrophilic drugs dispersed within a continuous oil phase. Their droplet size allows for a high surface area and solubilization capacity (Hamid et al., 2021). These emulsifier-stabilized systems can be used for the

delivery of drugs and enhanced controlled release. In this study, lipophilicity was identified as the primary determinant of drug release, with compounds exhibiting high logP values remaining longer within NE droplets. Synthesized NEs are characterized via DSC. Lipophilic prodrugs extended the release by increasing the drug loading capacity of NEs, while logP calculations and reversed-phase high-performance liquid chromatography analyses proved to be reliable predictors of drug release. These findings demonstrate that NE-based systems can be tailored for controlled drug release (Baumann et al., 2024). In another study, NEs were found to enhance the targeted and efficient delivery of antiretroviral drugs, such as darunavir and ritonavir, which are used in HIV treatment. Lipid-based nanoemulsions improve drug delivery to the intestinal lymphatic system, offering more efficient distribution than traditional therapeutic approaches. Their liquid-lipid structure enables high drug loading and strong colloidal stability (Elkateb et al., 2023).

3. Nanoparticle-Based Drug Delivery Systems

3.1. Challenges of Nanoparticle-Based Drug Delivery Systems

Nanoparticle-based drug delivery systems represent a promising area for targeted therapies; however, numerous challenges exist, including toxicity, biocompatibility, immunogenicity, limited tumor penetration, stability, protein corona formation, low drug loading capacity, insufficient cellular uptake, potential side effects, and physiological barriers (Sharma et al., 2025). Oral toxicity of Ag^{+} and $AgNP^{+}$ was investigated in rats. Despite similar tissue silver levels (1.4–1.6 μg/g), $AgNP^{+}$ demonstrated greater toxicity, leading to hair loss and

immunological dysfunction such as lymphocytosis. Raman spectroscopy indicated weak bone mineralization, while histology revealed spleen inflammation and anomalies in the bone marrow (Ezhumalai et al., 2025). The biocompatibility concerns of metal-organic frameworks (MOFs) have hindered their transition to clinical application. A machine learning-assisted computational pipeline to study MOF biocompatibility is presented. Using a database of more than 35,000 organic molecules, it predicts the toxicity of MOF binders in different application routes with an accuracy of higher than 80%. The model found hundreds of safe candidates and determined that Ag, Ca, and Zr were the most biocompatible metal centers after screening 86,000 MOFs. Additionally, it highlighted significant chemical characteristics that control toxicity, such as drug-like properties, which improve safety without decreasing porosity (Menon & Fairen-Jimenez, 2025). Several nanocarriers have been designed for tumor therapy. However, tumor delivery efficiency and off-target release concerns have posed a challenge in the transition of nanocarriers to clinical use. It is still a major challenge for nanocarriers to overcome multiple biological barriers simultaneously (Xue et al., 2025).

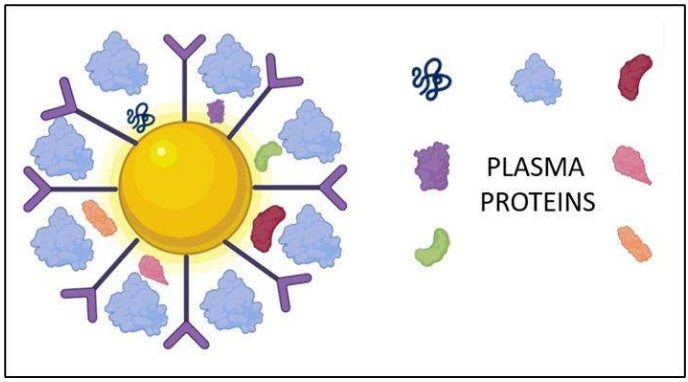

Figure 7: Illustration of protein corona structure (Created with BioRender.com).

One of the key challenges to be addressed in drug delivery systems is the protein corona formation (Figure 7). This structure forms in the bloodstream upon contact with the surface of nanoparticles and affects the circulation of nanoparticles in the body. Protein corona formation can cause triggering of immune cells, which can lead to premature clearance of nanoparticles from the body before they reach target cells (Kelle et al., 2025b). In the study, porous vaterite particles were used for sustained release in a controlled drug delivery system. Despite these challenges, such as low loading capacity and limited antibacterial properties, they hinder practical application. Vaterite particles synthesized at low temperatures were modified with stearic acid. This modified particle has a high loading capacity for doxorubicin as a cancer drug (Huang et al., 2025).

3.2. Advantages of Nanoparticle-Based Drug Delivery Systems

Besides the mentioned challenges, nanoparticle-based drug delivery systems also have various advantages, such as the ability to achieve targeting tumor cells, reducing side effects of drugs, enhancing physicochemical and biological stability, increasing specificity, non-invasive distribution, drug loading capacity, improving patient compliance, controlling drug release, and improving biocompatibility. Collectively, these characteristics allow reduce the frequency of dosages, keep an optimal drug concentration at the target site, and improve overall therapeutic efficacy. Additionally, by modifying their surfaces with ligands, biocompatible polymers, or lipid coatings, nanoparticle systems can decrease immune responses, increase circulation time, and enhance their safety profile. Furthermore, nanoparticle-based delivery systems enhance patient compliance, allow for sustained and site-specific release, and ultimately result in more effective and safer therapeutic responses (Prajapat et al., 2025; Wang et al., 2025).

Recently, Au/mesoporous silica nanoparticles have been designed for targeted tumor drug delivery. Au nanoparticles are functionalized with lactate oxidase, while silica particles are coated with α-cyclodextrin. The use of these nanoparticles also reduced the side effects of the drugs. Au nanoparticles showed increased specificity, and they accumulated in the mice's tumors (Zhang et al., 2025).

Transdermal drug delivery offers an alternative to both needle injection and oral medication administration because it improves patient compliance and convenience through a non-invasive delivery

method. The microneedle was produced in one study to deliver drugs deeply and quickly through the skin. As a result, the designed microneedles show a faster drug delivery, reducing the time efficiency compared to conventional microneedles (Chen et al., 2025).

On the other hand, a DNA dendrimer-based drug delivery system has been designed. According to the study, chemotherapeutic drugs have been transported and released under regulated conditions via DNA-based drug carriers that have been able to enter cancer cells by endocytosis and have broken down the DNA-based drug carriers when cellular glutathione levels are reduced. It has been shown that DNA dendrimer-based nanocarriers can carry several chemotherapy drugs with regulated release and targeted co-delivery (Tian et al., 2025).

Hairpin DNA is linked to Au nanoparticles for chemotherapy, gene therapy, and diagnostic applications. *In vitro* cytotoxicity tests demonstrated that hairpin DNA-functionalized Au nanoparticles exhibited effective gene therapy. Thus, hairpin DNA-functionalized Au nanoparticles presented a higher drug-loading capacity and powerful binding affinity for doxorubicin (DOX) compared to hairpin DNA (Liu et al., 2025).

4. Safety Tests

4.1. *In Vitro* and *In Vivo* Toxicity Tests

Many assays are used to evaluate *in vitro* toxicity, such as MTT, XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide), WST-1 (Water-Soluble Tetrazolium salt-1), Alamar Blue, LDH (lactate dehydrogenase), Annexin V/Propidium Iodide (PI),

Ames, micronucleus, neutral red uptake (NRU), acridine orange (OA) staining, terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL), bicinchoninic Acid (BCA) assays, Enzyme-Linked Immunosorbent Assay (ELISA), Immunohistochemistry (IHC), and western blotting. The MTT assay, first developed by Mosmann, is a colorimetric method for determining cell viability, proliferation, and cytotoxicity by measuring the metabolic activity of living cells. It is based on the ability of mitochondrial dehydrogenase enzymes in living cells to transform the yellow tetrazolium salt MTT into insoluble purple formazan crystals. These crystals are subsequently solubilized, typically using acidified isopropanol or DMSO, and measured spectrophotometrically at 570 nm (Mosmann, 1983). In recent applications, such as the evaluation of anticancer nanoemulsions, the MTT assay remains a typical method to determine cytotoxicity and IC_{50} values in both cancerous and non-cancerous cell lines (de Sousa et al., 2025).

The XTT assay is a colorimetric method used to determine cell viability and metabolic activity, which evaluates the reduction of the tetrazolium salt XTT into a water-soluble orange formazan product by mitochondrial enzymes in living cells. In contrast to MTT, XTT does not require a solubilization step, making it less labor-intensive and more stable (Comley & Turner, 1990).

The WST-1 assay is a colorimetric cell viability and cytotoxicity assay that uses cellular mitochondrial dehydrogenases in metabolically active cells to eliminate the tetrazolium salt WST-1. These enzymes convert WST-1 to a highly water-soluble formazan dye, which can be

quantified at 440-460 nm. The quantity of formazan dye produced is directly related to the number of live cells (Ishiyama et al., 1997). This assay provides a sensitive, simple, and non-radioactive approach for measuring drug-induced cell proliferation and cytotoxicity, as demonstrated in research using the A549 (human lung carcinoma) cell line treated with albumin-escin nanoparticles. It also benefits from developing a water-soluble formazan product that allows direct absorbance measurements without further solubilization (Munusamy et al., 2025).

The Alamar Blue assay is a non-invasive technique for measuring cell viability and metabolic activity that requires living cells to reduce resazurin to resorufin. This reduction, resulting in a fluorescent and colorimetric change, reflects cellular metabolic function and allows cell growth monitoring. In standard and hollow-fiber bioreactor cultures, the dye diffuses into the cells, is reduced, and then released into the media, allowing fluorescence measurements without directly sampling the cell compartment. The assay is sensitive enough to distinguish between different cell densities and is helpful for continuous or repeated measurements; however, if not removed quickly, it may cause reversible, time and concentration-dependent growth inhibition (Domińska et al., 2024; Gloeckner et al., 2001).

In the LDH Release Assay, LDH is released extracellularly when the integrity of the plasma membrane is disrupted by exposure to cytotoxic compounds. NAD^+ is reduced to NADH by the released enzyme, which also catalyzes the oxidation of lactate to pyruvate. The end product can be measured using colorimetric or spectrophotometric

techniques. (Sasaki & Ohno, 1994). The assay applies to both mammalian cells and parasites, allowing for the assessment of chemical toxicity or drug efficacy. It is considered sensitive for high-throughput screening, providing a direct assessment of cytolysis, different from metabolic or proliferation-based assays (Hawadak et al., 2023).

The Annexin V/PI assay is a flow cytometry technique that detects and quantifies apoptotic cells by focusing on phosphatidylserine (PS) externalization, a characteristic of early apoptosis. Annexin V, a calcium-dependent phospholipid-binding protein, specifically binds to PS exposed on the plasma membrane's outer layer during apoptosis. Cells in early apoptosis express Annexin V while refusing PI, a non-vital DNA dye that only penetrates cells with reduced membrane integrity (Koopman et al., 1994).

Ames *et al.* developed the Ames Test, a biological experiment that uses specially engineered *Salmonella typhimurium* bacteria to determine if a substance might cause mutations in DNA. These bacteria have mutations that make them unable to develop without histidine, an essential amino acid. If a chemical agent induces a reverse mutation that restores this ability, the bacteria form colonies on histidine-free plates, showing that the chemical is mutagenic. To make the test more sensitive, the bacterial strains were engineered to lack DNA repair and had abnormalities in their outer membrane, making them more susceptible to mutagens. This test is commonly used to evaluate compounds for possible cancer-causing properties (Ames et al., 1973). The micronucleus test is an *in vitro* genotoxicity assay that detects chromosomal damage by measuring micronuclei development in the

cytoplasm of cells that are in interphase, mainly human lymphocytes or lymphoblastoid TK6 cells. Cells are loaded with the drug with and without metabolic activation and then extracted, fixed, and stained for the microscopic observation of micronuclei in binucleated cells (Melzi et al., 2022; Rane et al., 2024).

NRU assay is a colorimetric test that evaluates cell viability and cytotoxicity by measuring living cells' ability to absorb and keep the neutral red dye in their lysosomes. Neutral red dye is a weak cationic dye that is absorbed by only living cells (Ates et al., 2017; Rodrigues et al., 2023). The OA staining assay measures cell viability and lysosomal membrane integrity by using OA's fluorescent color. OA can cross the cell membrane and diffuse into nucleic acids and specific organelles, such as lysosomes. Lysosomes show the red color, while nucleic acids are green under the fluorescent microscope (Eriksson et al., 2023; Lee & Rhee, 2025).

The TUNEL assay is a molecular technique that uses DNA strand breaks to identify and detect apoptotic cells. This method is frequently used in toxicology and cancer research to validate DNA damage related to apoptosis (Phull et al., 2021). The BCA assay is a colorimetric technique used to measure the amount of protein found in a total sample. It relies on proteins reducing Cu^{2+} to Cu^{+} under alkaline conditions, which is followed by the formation of a purple Cu^{+}–BCA complex that can be measured spectrophotometrically (Steć et al., 2022).

Flow cytometry is a high-throughput, laser-based biophysical method that allows for multiparametric research of the physical and

biological properties of cells or particles in solutions. Apoptosis tests allow for the quantitative and qualitative assessment of cell death by evaluating indicators such as phosphatidylserine externalization, mitochondrial membrane potential loss, and DNA content or fragmentation (Kumari et al., 2022).

ELISA is a laboratory test for detecting and quantifying antibodies or antigens in a sample. It works by attaching the target molecule to a plate and using an enzyme-linked antibody to produce an identifiable color change (Choi et al., 2016). IHC is a technique for detecting specific proteins in tissue samples. It uses antibodies that attach to the target protein, followed by a color reaction that shows where the protein is located in the tissue. Western blotting is a method for identifying individual proteins in a sample. Gel electrophoresis separates proteins by size before transferring them to a membrane and detecting the protein of interest via antibodies (Satofuka et al., 2025).

Bioluminescence imaging (BLI) is used to evaluate *in vivo* toxicity. BLI is a highly sensitive, non-invasive approach. BLI uses luciferase-catalyzed reactions of luciferin substrates to release photons for visualization of the biological processes within organisms in real time. BLI is highly beneficial for detecting tumor metastasis, biodistribution, and therapeutic efficacy due to its high signal-to-noise ratio, deep tissue penetration, and low background interference (Tian et al., 2024; Zhang et al., 2025).

4.2. Preclinical and Clinical Tests for Nanoparticle-Based Drugs

In recent years, the rapid advancement of nanotechnology has had a substantial impact on biomedical research, particularly in drug delivery, cancer therapy, and vaccine development. Various nanoparticles, including metallic, polymeric, carbon-based, and lipid-based systems, have been thoroughly tested for biocompatibility, cytotoxicity, and therapeutic efficacy. Understanding their safety and efficacy through *in vitro* and *in vivo* studies is vital for clinical application. Several studies investigated different nanoparticle platforms to determine their anticancer properties, targeted drug delivery possibilities, and overall safety profiles. This heading covers recent experimental results from cell-based and animal models, focusing on the relation between nanoparticle composition, biological interaction, and therapeutic performance.

Baskaran *et al.* used the MCF-7 (breast cancer) cell line to research the anticancer properties of AgNPs and AuNPs. The MTT test for cell viability showed that cells containing AgNPs had lower vitality (AgNP's IC_{50}= 50 µg/mL and AuNP's IC_{50}= 60 µg/mL). The results showed that toxicity is dose-dependent (Figure 8). To detect apoptosis, fluorescent acridine orange and propidium iodide dyes were used for dual staining. More apoptosis was found in AgNPs compared to AuNPs. According to the results of these two tests, AgNPs showed higher toxicity than AuNPs in the MCF-7 cell line, which showed that their anticancer efficacy was much stronger (Baskaran et al., 2024).

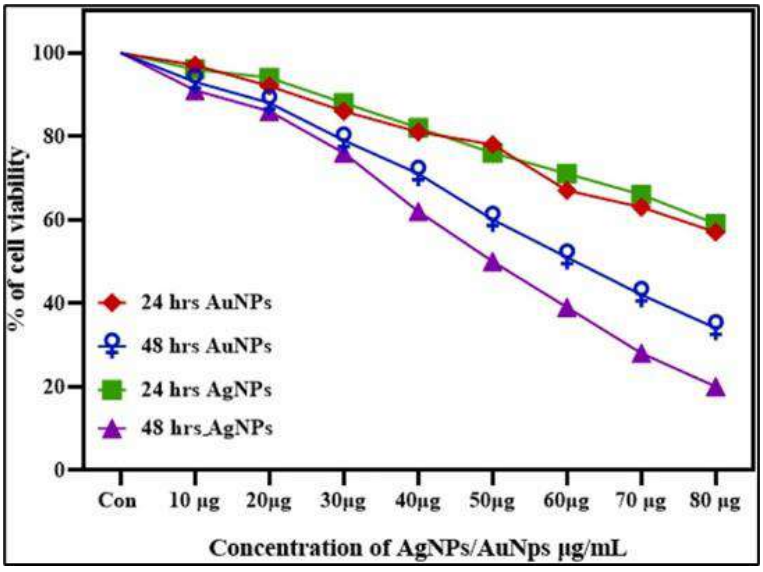

Figure 8: MTT test results that show the dose-dependent toxicity. Au nanoparticles and Ag nanoparticles effects on cell viability are dose- and time-dependent. Au nanoparticles, 24 h, red diamonds; 48 h, blue circles, and Ag nanoparticles, 24 h, green squares; 48 h, purple triangles were added to cells at progressively higher concentrations (10–80 µg/mL). Viability was set at 100% for the untreated control (Baskaran et al., 2024).

Liu et al. studied the safety of Fe_3O_4@C/ZnO-DOX-FA nanoplatforms in vivo experiments. In vivo tests on tumor-bearing mice showed effective tumor suppression, with the DOX-loaded, FA-targeted, laser-irradiated group reaching 90% inhibition. Histopathological testing of major organs showed negligible tissue damage, and biodistribution tests confirmed tumor-specific accumulation with negligible off-target results. No weight loss or

systemic toxicity was observed, and the treatment group had the highest survival rate, indicating high biocompatibility and safety (Liu et al., 2021).

Targazeh *et al.* used the Soas-2 (osteosarcoma) cell line for research on the use of polyglycerol graphene oxide (PGO) for enhancing DOX loading capacity. The MTT test for cell viability showed that DOX-loaded PGO was more toxic than bare PGO. Annexin V/PI staining and flow cytometry were used to detect apoptosis, and the results showed that dox-loaded PGO had more apoptosis. (Figure 9) *In vivo* experiments showed that DOX developed structural damage, whereas no significant tissue damage was observed in DOX-PGO or PGO (Targhazeh et al., 2023).

Tan *et al.* studied using PEG-phospholipid functionalized carbon nanotubes to enhance siRNA systemic delivery. Human cell lines MCF7, HEL-293T (Human embryonic kidney), H1299 (Human non-small cell lung carcinoma), and HeLa were utilized in this research. The LDH Release Assay was employed to evaluate cytotoxicity, with results indicating non-significant LDH release. Luminescence screening was conducted on H1299-Luc cells transfected with siLuc to assess cell viability, which showed no significant change in luminescent signal. *In vivo* toxicity tests were performed on mouse models by administering a dosage of 0.8 mg/kg intravenously via tail vein injection. Results indicated no significant systemic toxicity (Tan et al., 2024).

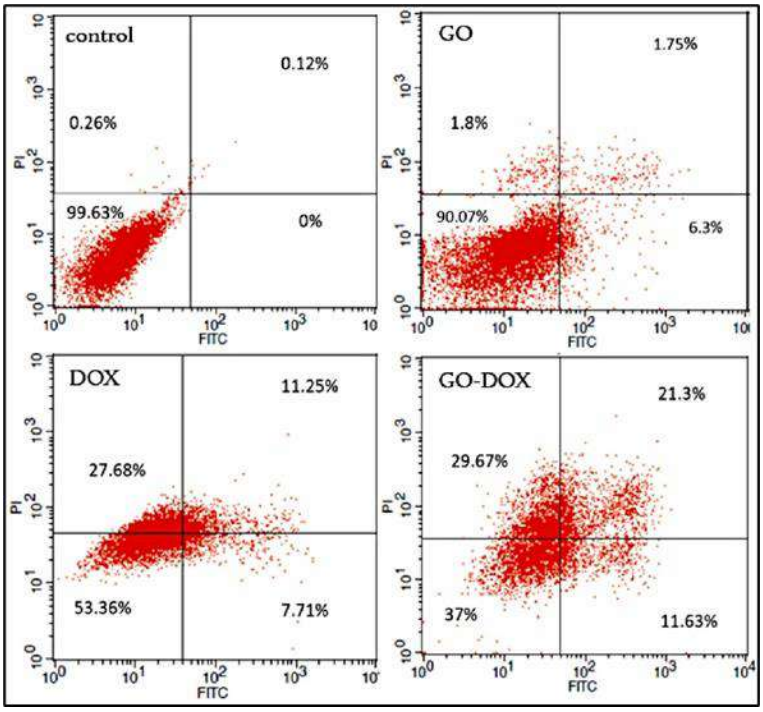

Figure 9: Annexin V/PI stained and flow cytometry chart that indicates the apoptosis. Viable cells are represented by the lower left quadrant; early apoptosis is indicated by the lower right; late apoptosis or necrosis is indicated by the top right; and necrotic cells are represented by the upper left. The metabolic activity of control cells was 99.63% (Targhazeh et al., 2023).

Zhang *et al.* evaluated the effect of PLGA nanoparticles in a targeted delivery system using RAW264.7 macrophages and splenic lymphocytes isolated from mice. Cell proliferation tests were conducted using flow cytometry and fluorescence labeling methods. Both the cell lines tested showed no cytotoxic effects. The main organs, such as the heart, liver, spleen, lungs, and kidneys, were

histopathologically tested for *in vivo* tests. The results did not show significant organ toxicity. Similarly, BLI results showed no systemic toxicity or tissue damage (Zhang et al., 2024).

In the study by Binici *et al.,* the effect of PEGylated LNPs on transfection efficiency and cytotoxicity was systematically assessed using HEK293. The cells were transfected with firefly luciferase (FLuc) mRNA-loaded LNPs at doses of 25, 50, and 100 ng per well. Cell viability was measured using the Alamar Blue assay after 24 h of incubation, showed minimal cytotoxicity across all concentrations (Figure 10). Transfection efficiency was assessed by measuring FLuc expression levels. For *in vivo* analysis, 5 µg of FLuc mRNA-loaded LNPs were intramuscularly injected into both legs of mice, and protein expression was measured using BLI. The results showed that PEGylated LNPs enhanced protein expression with negligible toxicity (Binici et al., 2025).

Broudic *et al.* moved from *in silico* to *in vitro* tests to examine the safety of LNPs in mRNA-based vaccines. The Ames test was conducted with five different bacterial strains, and the results showed no toxicity but a slight increase in cell viability. The micronucleus test used micronuclei as a biomarker, with the TK6 (human lymphoblast cells from the spleen) cell line used. The results showed no significant increase in micronucleated cells at any dose. *In vivo* experiments, mRNA-loaded LNPs encoding influenza-specific antigen were injected intramuscularly into female rabbits at doses of 0, 10, 50, 125 250, 250, and 500 µg. After 2 weeks, no mortality or systemic toxicity was observed. There were mild weight loss and fever, thought to be related

to the high dose. Histopathological tests showed a small amount of acute inflammation and no severe systemic organ toxicity (Broudic et al., 2022).

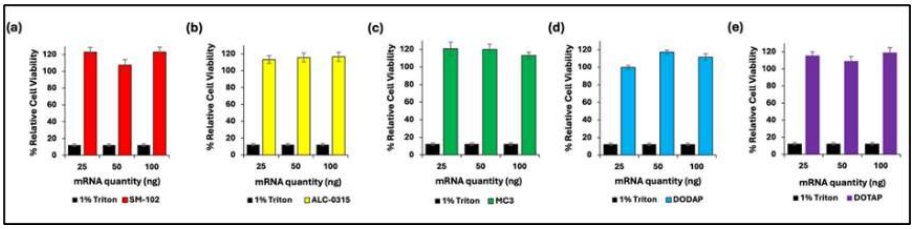

Figure 10: Alamar blue assay results for different types of LNPs. Following administration of LNPs containing (a) SM-102, (b) ALC-0315, (c) MC3, (d) DODAP, and (e) DOTAP at mRNA amounts of 25, 50, and 100 ng, cell viability was tested. Complete cell lysis is indicated by the black bars, which stand for the positive control (1% Triton X-100). At all mRNA doses, all LNP formulations showed robust cell survival (higher than 90%) (Binici et al., 2025).

In another study, a whole blood assay was used to examine LNPs for immune activation and cytokine release. Blood samples were collected from both healthy and systemic lupus erythematosus (SLE), type 2 diabetes mellitus (T2DM), and cancer donors, and while leukocytes showed low expression, myeloid cells showed high expression compared to other cells. Marker-based assays were used for immune cell activation tests, and the findings showed that empty LNPs did not affect immune cell activation, although certain empty LNPs were found to induce cytokine release (Nguyen et al., 2024).

Bae *et al.* conducted *in vitro* experiments using HepG2, HEK293, and fibroblast cell lines to test the safety of mRNA LNP vaccines. Even at high doses (2mM), higher than 80% cell viability was measured, which indicated no toxicity. This study showed that LNPs are very biocompatible. *In vivo*, toxicity tests have been conducted in mice using intramuscular injection of mRNA LNPs and BLI (Figure 11). It showed that trehalose glycolipid-containing LNPs reduced liver and spleen toxicity even at high doses (Bae et al., 2024).

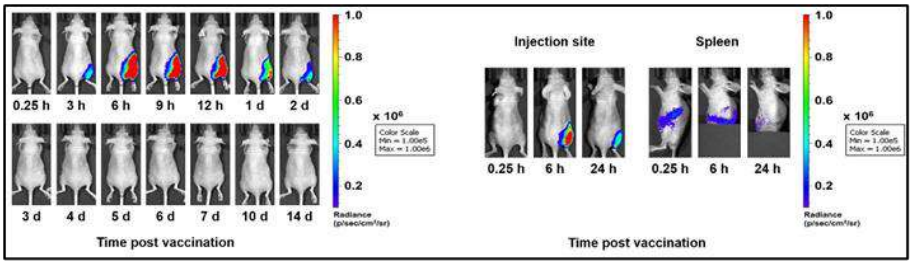

Figure 11: BLI results of the temporal expression of the mRNA at the injection site are demonstrated. The signal strength peaks between 6 h and 12 h and then steadily decreases by day 3, becoming undetectable by day 7. Strong luciferase expression at the injection site and subsequent migration to the spleen are highlighted by region-specific imaging. Expression was visible at 6 h and decreased by 24 h after injection (Bae et al., 2024).

Dutta *et al.* conducted *in vitro* analyses on the toxicity of curcumin-loaded PEGylated LNPs using the A549 and W126 (human lung epithelial) cell lines. The MTT cell viability assay showed that the A549 cell line had significant dose-dependent cytotoxicity; however,

the W126 cell line had a low level of cytotoxicity. For *in vivo* tests, mice were injected intravenously at a 200 mg/kg/day dose after 28 days. The histopathologic test results showed no tissue damage in the liver, kidney, and lung. (Figure 12) In conclusion, curcumin-loaded PEGylated LNPs have the potential to be used for the treatment of lung cancer (Dutta et al., 2024).

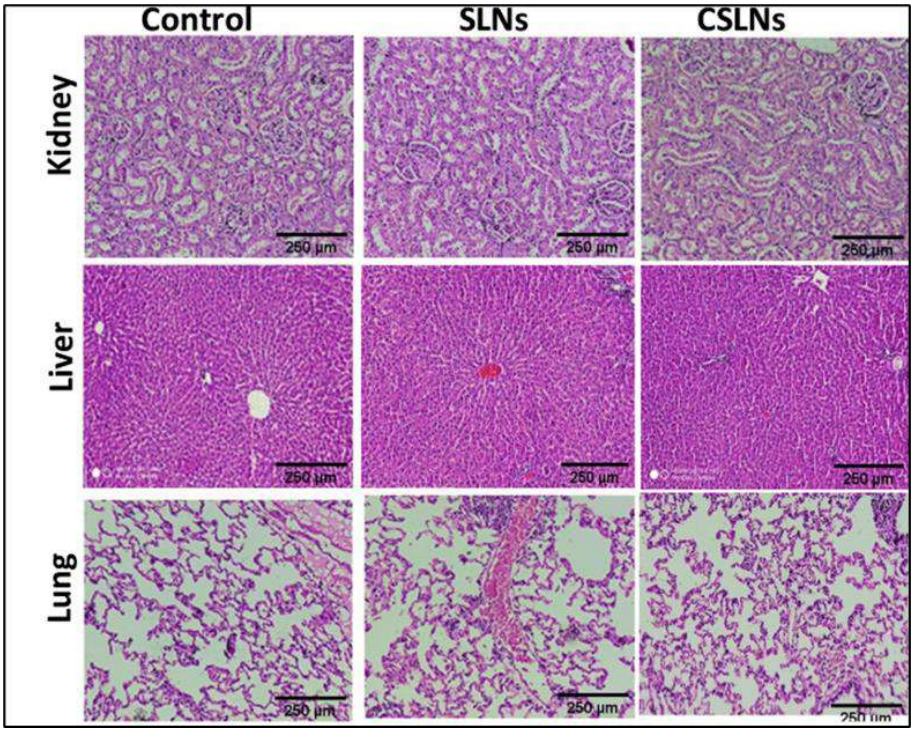

Figure 12: Histopathological analysis results. Light microscopy (scale bar = 250 µm) was used to evaluate tissue slices of the kidney, liver, and lung from the control, SLN, and curcumin-loaded SLN treatment groups that had been stained with hematoxylin and eosin (H&E) (Dutta et al., 2024).

Tatovik *et al.* evaluated the safety of proinsulin peptide conjugated with Au nanoparticles in a Phase I trial involving six volunteers, with five completing the analysis. Participants received three-month intradermal injections via microneedles. Local injection site reactions were observed without systemic adverse events, supporting the safety of Au nanoparticles (Tatovic et al., 2022).

Hao *et al.* conducted a clinical study involving 100 patients, assessing carbon nanoparticles for lymph node mapping in papillary thyroid microcarcinoma. Intradermal injections resulted in no local or systemic reactions, demonstrating superior accuracy and safety compared to methylene blue in nanoparticle form. It exhibited a favorable safety profile in Phase II trials for recurrent ovarian cancer, with mild-to-moderate nausea, fatigue, and anemia as the most frequent adverse events (Hao et al., 2012). Severe toxicities were rare, and no treatment-related deaths occurred. The addition of bevacizumab increased hypertension and bladder toxicity rates but did not result in significant adverse outcomes (Krasner et al., 2021). Polack *et al*. conducted a randomized experiment with 43,448 volunteers to assess the safety and efficacy of the COVID-19 vaccine. Among subjects tested longer than seven days after the second dose, eight COVID-19 instances occurred in the vaccine group against 162 in the control group, resulting in 95% efficiency. High efficacy was consistently reported in all demographic and clinical subgroups. Of the ten severe COVID-19 cases after the first dosage, nine were placebo recipients and one was a vaccination recipient. This vaccine had a positive safety profile, with mild-to-moderate local and systemic reactions, and the

incidence of significant adverse events was low and comparable between groups (Polack et al., 2020).

4.3. Biocompatibility

4.3.1. Biocompatibility of Nanoparticle-Based Drugs

Biocompatibility is crucial in the research and clinical application of nanoparticle-based drugs. Nanoparticles interact directly with cells, tissues, and biological fluids. Their composition, surface characteristics, and stability all affect how safely and effectively they can deliver therapeutic compounds. By achieving high biocompatibility, nanoparticles are able to function as intended therapeutic agents without causing cytotoxic effects, hemolysis, or immunological responses. Consequently, scientists are currently focusing on developing nanoparticles that combine stability, biodegradability, and specific cellular interactions; consistently, this occurs by surface functionalization. Assessing parameters such as protein adsorption, hemocompatibility, and cell viability offers crucial details on their biological safety. Developing safe, effective, and clinically beneficial nanoparticle-based drugs requires an understanding of and dedication to improving these characteristics.

Ezhumalai *et al.* synthesized *N*-acetylcysteine functionalized cholic acid-based tri-armed poly(Dl-lactide), a biocompatible polymer. This polymer formed reverse polymeric micelles. They synthesized this polymer to stabilize Au nanoparticles. The hemolysis test was performed to test biocompatibility. The results showed biocompatibility even at high doses (512 μg/ml). Antioxidant activity was found to be

dose-dependent. DOX loading was performed for drug release, and controlled drug release was observed. The combination of biodegradable polymer and Au nanoparticles showed promising results for safer drug delivery (Ezhumalai et al., 2024).

Uzoeto *et al.* investigated the biocompatibility of zinc oxide nanoparticles produced by the green synthesis method. Human red blood cells were used for a hemolysis test, and the percentage of hemolysis was calculated to be lower than 5%. The production of zinc oxide nanoparticles by green synthesis increased stability, biocompatibility, and antioxidant activity (Uzoeto et al., 2024).

Chatterjee *et al.* synthesized chitosan and magnet-based nanoparticles and 5-fluorouracil. The MTT test was performed using the MDA-MB-231 (breast cancer) cell line. The results showed a decrease in cell viability. TEM, SEM, and AFM confirmed the spherical and porous structure, and this also increased the drug loading capacity. The pH sensitivity observed in the drug release profile was a critical element for cancer targeting (Chatterjee et al., 2025).

On the other hand, Puvvada *et al.* functionalized the surface of CNPs with folic acid. This is attributed to targeted therapy in breast cancer cell lines (MCF-7 and T47D). MTT and hemolysis assays were performed to assess biocompatibility, and the findings revealed enhanced cytotoxicity and apoptosis. There was no cytotoxicity in healthy cells. CNP's pH sensitivity (pH=5.2 in breast cancer) supports targeted cancer therapy (Puvvada et al., 2024).

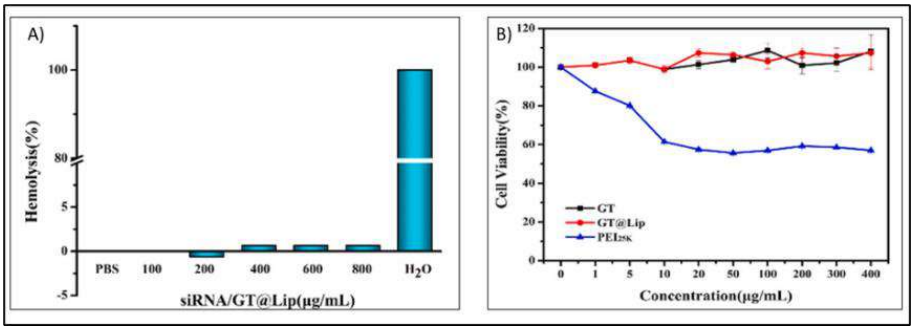

Figure 13: (A) In the hemolysis assay, PBS and H_2O are used as negative and positive controls, respectively, to show the percentage of hemolysis caused by siRNA/GT@Lip formulations at increasing concentrations (100–800 μg/mL). MTT assay contrasting formulations of GT, GT@Lip, and PEI25k over a concentration range of 0–400 μg/mL (Chen et al., 2024).

Khalifeh *et al.* investigated the mRNA delivery of lipid-coated calcium phosphate nanoparticles. In cytotoxicity tests, it was observed that PEGylation reduced the toxicity, while in the hemocompatibility test, it increased cell viability. Protein corona formation and endosomal escape were examined for the mRNA delivery test, and it was shown that the nanoparticle was stable and escaped from the endosome without degradation (Khalifeh et al., 2025).

Another study showed that glycogen-based nanoparticles were coated with a lipid bilayer, and siRNA delivery was examined in the NIH3T3 cell line. Hemolysis and MTT test for biocompatibility, protein adsorption stability, and endosomal escape tests were performed (Figure 13). The results showed lower than 1% hemolysis percentage, higher than 85% cell viability, and high stability even at high doses; no

protein corona was observed, and endosomal escape occurred before degradation (Chen et al., 2024).

4.3.2. Enhancing Biocompatibility and Applications

Many methods can be used to enhance the biocompatibility of nanoparticles. Methods such as coating with natural polymers or cationic lipids, surface modifications such as PEGylation, production methods such as green synthesis, and the use of hybrid nanoparticles can be applied to enhance biocompatibility. Natural polymer coatings are widely used to improve nanoparticle biocompatibility and stability. These biopolymers form a protective layer on the nanoparticle surface, reducing non-specific protein adsorption and immune recognition. For instance, chitosan-coated magnetic nanoparticles have been shown to exhibit improved dispersion in physiological media and relatively low cytotoxicity (Zoe et al., 2023). Cationic lipids such as dimethyldioctadecylammonium bromide (DDAB) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) are widely used in lipid-based nanocarriers to enhance effectiveness and cell membrane interaction. When modified, these lipids positively charged surface allows for electrostatic interaction with negatively charged biomolecules such as proteins, RNA, or DNA, improving cellular uptake while providing an acceptable level of cytocompatibility (Ochoa-Sánchez et al., 2024; Sun & Lu, 2023). Furthermore, one of the effective methods for improving biocompatibility is surface modification via PEGylation. PEGylation has effects on increasing circulation time *in vivo* and reducing

reticuloendothelial system (RES) recognition and clearance (El-Baz et al., 2022).

Although biocompatibility and toxicity tests are critical in preventing adverse effects, they are also important for fundamentally understanding the relationship between the physicochemical properties of nanomaterials and their specific biological effects. They also play a major role in expanding the range of applications. These tests are also critical for ensuring the safety of patients. Additionally, biocompatibility involves a comprehensive evaluation of nanoparticles *in vivo* and *in vitro*. Therefore, it is necessary to reduce cytotoxicity and enhance biocompatibility (Kyriakides et al., 2021).

Zia *et al.* used the L929 (murine fibroblast) cell line to study the carbon coating of Ag nanoparticles. The Alamar Blue Assay was used for biocompatibility studies, and cell viability was investigated. Diamond-like carbon was shown to be highly biocompatible due to its natural origin, but Ag nanoparticles had a significant cytotoxicity. Diamond-like carbon-coated Ag nanoparticles reduced the cytotoxicity and were found to be highly biocompatible. Similarly, a 63% increase in hardness and a 100% increase in Young's modulus were observed (Zia et al., 2024).

Manzoar *et al*. used HTC-116 (human colon cancer) and human red blood cell lines in their study on green-synthesized (*Trillium govanianum*) Ag nanoparticles. They aimed to increase biocompatibility by coating Ag nanoparticles with phytochemicals. Hemolysis and MTT test were applied for cytotoxicity tests, and the

results showed 84% inhibition in the HTC-116 cell line, while the hemolysis rate was 25% in red blood cells (Manzoor et al., 2025).

Xiao *et al.* studied anti-deep vein thrombosis therapy using rat red blood cells, rat neutrophils, HUVEC, and HEK-293A. Mesoporous silica nanoparticles were used in the study due to their high drug loading capacity and biocompatibility. For higher biocompatibility, nanoparticles were coated with a platelet membrane. Hemocompatibility and cell viability were examined for safety tests. While hemocompatibility test results showed lower than 5% hemolysis, coated silica nanoparticles reduced the hemolysis rate. In cell viability results, coated silica nanoparticles showed a higher survival rate in HUVEC and HEK-293A cell lines (Xiao et al., 2024).

Yen *et al.* studied terpolymer-lipid-MnO_2 nanoparticles used in the cell lines U87MG-luc (glioblastoma), PC3 (prostate cancer), NIH3T3 (murine fibroblast), and MCF10A (human epithelial). MTT assay was performed on NIH3T3 and MCF10A cells for cytotoxicity, and the results showed higher than 90% cell viability. U87MG-luc and PC3 cell lines were used for tumor targeting, and tumor-specific activation was observed. Histopathological tests and BLI were used for *in vivo* safety, and the results showed no Mn accumulation. A terpolymer-lipid coating made MnO_2 particles stable (Yen et al., 2024).

Wu *et al.* used Span-80 modified lipid nanocapsules in the B16F10 (mouse melanoma) cell line. Span-80 polymer was modified into lipid nanocapsules to increase biocompatibility. In cytotoxicity tests, Span-80 modified lipid nanocapsules showed lower toxicity. When protein corona formation was investigated, the hydrophobicity of

Span-80 enhanced protein interaction and increased bioactivity (Wu et al., 2025).

Santhanes *et al.* used HEK293T for *in vitro* tests and a mouse model for *in vivo* tests in their study on the design of lipid-polymer hybrid nanoparticles. They used cationic lipids and polymers for nanoparticle design and PEG for surface modification. MTT assay was performed for cell viability, and the results showed 60-100% viability in RS100 polymer-based nanoparticles, while E100 polymer-based nanoparticles showed lower levels of viability. These polymers were used due to their pH-responsive structure, but at low doses, E100-based nanoparticles were more efficient as they dissolved at a pH lower than 5. Likewise, PEGylation increased gene delivery efficiency. *In vivo* tests supported the results of *in vitro* tests (Santhanes et al., 2024).

Taheri *et al.* used green-synthesized carbon quantum dots in MDA-MB-231 (human breast cancer) and HDF (human dermal fibroblast) cell lines. Taheri et al. performed hemocompatibility and MTT tests and found that quantum dots specifically targeted cancer and decreased cell viability, while healthy cell lines showed high cell viability. The effect of the synthesis method of the nanoparticles on biocompatibility was observed (Taheri et al., 2025).

Li *et al.* studied CNT-chitosan nanoparticle hybrids by combining CNTs with natural polymer chitosan nanoparticles to reduce CNT's toxicity. As a result of the MTT test in the HeLa cell line, cell viability was 81% even at high doses (100 µg/mL). In the MTT test of CNTs alone, the viability was 54% at 100 µg/mL. In the BSA test for protein

immobilization, this rate was 42.8% in CNTs and 76.8% in CNT-chitosan nanoparticle hybrids (Li et al., 2011).

4.4. Interaction Between Biological Systems

In the 1980s, Vroman *et al.* discovered that when any synthetic material, including nanoparticles, encounters a biological fluid, it is rapidly coated by proteins. This discovery is considered the first interaction between nanoparticles and biological environments (Vroman et al., 1980). This protein-coated state of nanoparticles is called "protein corona" and gives the nanoparticle a new biological identity (Figure 6). In fact, most particles fail to reach their target and accumulate in off-target organs such as the liver, spleen, and lungs (Rampado et al., 2020).

Ahmadi *et al*. used MCF-7 and HFF-1 (human foreskin fibroblast) cell lines and the BALB/c mouse model in their study on *in vivo* pharmacokinetics and biodistribution of technetium-99 m-labeled Zn-based MOFs. The nanoparticles were characterized by DLS, FTIR, SEM, and TEM, and their cytotoxicity was examined by *in vitro* MTT assay. Up to 80 µg/mL doses did not show significant cytotoxicity. The *in vivo* results showed the desired accumulation in the lung. Area Under the Curve (AUC (%ID-h/g)), half-life (t½ (h)), clearance (mL/h), volume of distribution (Vd (mL)), and mean residence time (MRT (h)) were analyzed for pharmacokinetic analysis because of the presence of protein corona formation. The results showed that the nanoparticles had prolonged circulation, slower clearance, and enhanced systemic exposure (Ahmadi et al., 2023).

Ali *et al.* used a TiO_2/CNTs-modified carbon paste electrode in rabbit plasma to investigate the pharmacokinetic interaction between DOX and avanafil (AVN). XRD, FTIR, and SEM were used for characterization. AVN was applied to one group, whereas the other group received both AVN and DOX. Non-compartmental analysis was used to determine the pharmacokinetic parameters. The combined administration of DOX resulted in a substantial increase in the $t^{1/2}$, AUC, and AVN plasma concentration. The presence of protein corona formation caused an increase in these parameters. The approach indicated AVN dosage modification during combined administration and showed that DOX changed AVN pharmacokinetics (Ali et al., 2023).

Tian *et al.* investigated the pharmacokinetics and biodistribution of Au nanoparticles coated with either monodisperse (PEG36, PEG45) or polydisperse PEG using BALB/c mice that had CT26 tumors. TEM, DLS, and TGA were used to characterize the nanoparticles. The BSA method was used to test protein adsorption. Protein binding was significantly reduced by monodisperse PEG-Au nanoparticles. ICP-MS was used to analyze the liver, spleen, blood, and tumor tissues for biodistribution. In contrast to polydisperse PEG-Au nanoparticles, pharmacokinetic assay data demonstrated that PEG36-AuNPs showed a longer $t^{1/2}$ and higher tumor accumulation, indicating enhanced circulation and targeting monodisperse PEG (Tian & Yuan, 2024).

Javed *et al.* investigated the *in vivo* pharmacokinetics and ocular retention of thiolated chitosan nanoparticles loaded with tobramycin using a sheep model and the ARPE-19 (human retinal pigment

epithelial) cell line. TGA, DLS, FTIR, XRD, and DSC were used to characterize the nanoparticles. The MTT assay was used to determine cytotoxicity, and cell viability was found to be higher than 80%. AUC, t½, clearance, and MRT were among the pharmacokinetic parameters that were examined. Tobramycin-loaded nanoparticles showed enhanced ocular retention, a 5-fold reduction in clearance, and a 1.5-fold increase in AUC when compared to free tobramycin. The results showed improved ocular bioavailability, decreased toxicity, and prolonged drug release (Javed et al., 2023).

Dave *et al.* investigated the pharmacokinetics and biodistribution of camptothecin-loaded hybrid lipid-polymer nanoparticles composed of PLGA and DSPE-mPEG2000 using Sprague-Dawley rats and the SW-620 (colorectal cancer) cell line. DLS, TEM, DSC, and XRD were used to characterize the nanoparticles. The MTT assay was used to assess cytotoxicity, and the results showed improved efficacy with a lower IC_{50} than free camptothecin. With a 3-fold increase in $t^{1/2}$, a 3.6-fold increase in AUC, an extended MRT, and reduced clearance, these nanoparticles showed significant enhancement in pharmacokinetic studies. The results showed improved systemic circulation, increased tumor targeting, and prolonged drug release because of the presence of protein corona formation (Dave et al., 2025b).

Walter *et al*. investigated the biodistribution and pharmacokinetics of lipid–PLGA hybrid nanoparticles designed as an oxycodone vaccination using BALB/c mice. After PLGA and lipids were combined, oxycodone-protein conjugates that were used as carrier proteins were attached to form the nanoparticles, which had a size of

about 150 nm. The strongest antibody response, the lowest oxycodone levels in the brain, and the highest blood levels have been observed with this vaccine. The results showed that this nanoparticle vaccination provided enhanced safety compared to conventional vaccinations, enhanced drug circulation, and decreased oxycodone passage into the brain (Walter et al., 2025).

Chettupalli *et al*. investigated the pharmacokinetics and pharmacodynamics of empagliflozin-loaded SLNs in streptozotocin-induced diabetic Sprague-Dawley rats. Ultrasonication was used to produce the SLNs, and DLS, SEM, TEM, AFM, FTIR, DSC, and XRD were used to characterize SLNs. The optimized formulation showed a high entrapment efficiency (90.6%) and a particle size of 98.6 nm. Comparing SLNs to the drug suspension, the pharmacokinetic study showed that these nanoparticles had a 2.2-fold higher AUC, a 3.7-fold longer $t^{1/2}$, and a lower clearance. *The in vitro* release showed sustained drug release up to 48 h. Results showed that empagliflozin-SLNs increased oral bioavailability, extended circulation, and improved systemic exposure (Chettupalli et al., 2025).

5. Ethics

The expeditiously developing field of nanoparticle-based drug delivery raises many ethical issues that need to be addressed in addition to their potential uses in medicine. In biological environments, these systems exhibit unique characteristics that raise ethical and societal issues besides toxicological and pharmacokinetic concerns.

First, it's still not quite understood how nanoparticles will affect human health in the long term. The process becomes a fundamental ethical requirement in clinical studies when taking into consideration the potential of bioaccumulation, immunological modulation, and unexpected cellular interactions. The nature of the nanoparticles, the characteristics of the carrier systems, possible toxicities, and unidentified concerns must all be clarified to the participants. To develop and carry out well-controlled clinical trials that adhere to ethical standards and regulatory requirements, interaction between academia, industry, and patient advocacy organizations is essential.

Nanoparticle-based drugs are frequently assessed for safety using animal models, such as zebrafish, mice, and rabbits. In these preclinical tests, maintaining ethical consistency is essential. To minimize the usage of animals, studies should be designed using the 3Rs Principle (Replacement, Reduction, and Refinement) (Díaz et al., 2021) and, when possible, alternative techniques such as 3D cell culture systems.

Sustainability of the environment is another crucial ethical consideration. Because of their resistance to degradation and tendency to accumulate in soil or aquatic systems, Metal-based and carbon-based nanoparticles may pose ecological issues. Consequently, using green synthesis and other environmentally friendly production techniques is a scientific and ethical responsibility.

Furthermore, the production and application of nanoparticle-based drugs depend on sophisticated technologies that are expensive and resource-intensive. When wealthy countries have access to these therapies and countries with low incomes have to miss out, this may

increase global health inequalities. For instance, the cancer drug nab-paclitaxel has not been extensively employed in the treatment of breast cancer patients in China. The primary cause of this, its high cost (about $6,200 for four sessions). Nab-paclitaxel has been listed for large-scale central procurement since January 2020, making it more accessible to patients (about $1,700 for four sessions) (Lu et al., 2021). Another example that shows the ethical importance of equal access to nanotechnology-based therapies is the unequal distribution of mRNA-based vaccinations during the COVID-19 pandemic.

Finally, most of the safety and biocompatibility information given by current research is preliminary, and longer-term, comprehensive investigations are still essential. Consequently, nanoparticle-based drugs should ensure that their development and application respect ethical principles before entering clinical use.

6. Future Prospects

Nanoparticle-based drugs have significant potential to improve precision medicine, treatments, and diagnostics in the future. The development of more creative, stimulus-responsive, and disease-targeted nanoparticles is expected to go beyond present limitations such as systemic toxicity, low bioavailability, and biological barrier permeability as nanotechnology grows and develops. Future studies in this area are most likely to concentrate on creating more precise and scalable techniques for synthesizing nanoparticles with controlled surface properties, size, and shape. Specifically, by determining the most optimal physicochemical characteristics, reducing experimental

effort, and minimizing trial-and-error procedures, the integration of machine learning into nanoparticle design and safety prediction will significantly speed up the drug development pipelines. In the future, surface engineering techniques will likely be crucial for reducing immunological recognition, increasing the circulation of nanoparticles, and enhancing target effectiveness. To ensure a safe transition from laboratory to patients, it will be crucial for regulatory sciences to integrate global regulations related to nanoparticle-based drugs. It is expected that high-throughput screening and omics-based toxicology techniques would significantly improve comprehensive safety testing employing *in vitro* and *in vivo* techniques. Animal models will likely be replaced by more accurate and predictive *in vitro* and *in vivo* models, such as organ-on-chip and 3D-bioprinted systems, which will increase beneficial applications. The gap between laboratory success and commercial viability will also be reduced with further efforts to scale up production techniques while ensuring quality control. The next decade is projected to see a paradigm change toward safer, smarter, and more accessible nanoparticle-based treatments, with applications in cancer, infectious diseases, gene therapy, and beyond.

CONCLUSION

A breakthrough in pharmaceutical sciences, nanoparticle-based drugs provide novel solutions to the ongoing problems of conventional drug formulations, such as weak solubility, rapid degradation, limited target selectivity, and systemic toxicity. Numerous studies have methodically investigated the production, characterization, and therapeutic applications of different kinds of nanoparticles as well as their incorporation into advanced drug delivery systems. To improve drug stability, bioavailability, and cellular absorption, these nanosystems offer adaptable physicochemical characteristics, such as size, surface charge, shape, and functionalization. The evaluation of biocompatibility and safety is a crucial area of focus in the development of these systems, as these factors are still crucial for their successful clinical application. PEGylation, lipid or polymeric coatings, and green synthesis techniques are examples of modification strategies that have been shown *in vitro* and *in vivo* to significantly decrease toxic effects, reduce immune response, and improve pharmacokinetic characteristics. Concerns like immunogenicity, inappropriate accumulation, protein corona formation, and the potential of long-term toxicity still exist despite all of this. These issues indicate the requirement for detailed safety tests using biodistribution studies, histopathology analyses, and toxicity tests such as MTT, LDH, and flow cytometry. According to current studies, many nanoparticle systems can achieve targeted delivery and reduced systemic toxicity with proper surface modifications and control of dosages. Simultaneously, ethical and

regulatory factors are increasing in importance. Environmental concerns might accelerate the adoption of alternative models such as 3D cell cultures and organ-on-a-chip systems. Green synthesis techniques offer safer and more environmentally friendly alternatives. To sum up, nanoparticle-based drugs have the potential to completely change current medicine, especially when it comes to targeted therapies. Overcoming current limitations, improving safety profiles, and converting innovations into affordable, clinically effective pharmaceuticals will all depend on sustained multidisciplinary collaboration.

REFERENCES

Adelantado, C., Lapizco-Encinas, B. H., Jordens, J., Voorspoels, S., Velimirovic, M., & Tirez, K. (2024). Capillary Electrophoresis as a Complementary Analytical Tool for the Separation and Detection of Nanoplastic Particles. *Analytical Chemistry*, *96*(19), 7706–7713. https://doi.org/10.1021/ACS.ANALCHEM.4C00822/ASSET/IMAGES/LARGE/AC4C00822_0004.JPEG

Afzal, O., Altamimi, A. S. A., Nadeem, M. S., Alzarea, S. I., Almalki, W. H., Tariq, A., Mubeen, B., Murtaza, B. N., Iftikhar, S., Riaz, N., & Kazmi, I. (2022). Nanoparticles in Drug Delivery: From History to Therapeutic Applications. *Nanomaterials*, *12*(24). https://doi.org/10.3390/NANO12244494

Ahmad, S., Majeed, M. I., Nawaz, H., Javed, M. R., Rashid, N., Abubakar, M., Batool, F., Bashir, S., Kashif, M., Ali, S., Tahira, M., Tabbasum, S., & Amin, I. (2021). Characterization and prediction of viral loads of Hepatitis B serum samples by using surface-enhanced Raman spectroscopy (SERS). *Photodiagnosis and Photodynamic Therapy*, *35*, 102386. https://doi.org/10.1016/J.PDPDT.2021.102386

Ahmadi, M., Khoramjouy, M., Dadashzadeh, S., Asadian, E., Mosayebnia, M., Geramifar, P., Shahhosseini, S., & Ghorbani-Bidkorpeh, F. (2023). Pharmacokinetics and biodistribution studies of [99mTc]-Labeled ZIF-8 nanoparticles to pave the way

for image-guided drug delivery and theranostics. *Journal of Drug Delivery Science and Technology*, *81*, 104249. https://doi.org/10.1016/J.JDDST.2023.104249

Aldeen, T. S., Ahmed Mohamed, H. E., & Maaza, M. (2022). ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. *Journal of Physics and Chemistry of Solids*, *160*, 110313. https://doi.org/10.1016/J.JPCS.2021.110313

Ali, A. M. B. H., Rageh, A. H., Abdel-aal, F. A. M., & Mohamed, A. M. I. (2023). Anatase titanium oxide nanoparticles and multi-walled carbon nanotubes-modified carbon paste electrode for simultaneous determination of avanafil and doxorubicin in plasma samples. *Microchemical Journal*, *185*, 108261. https://doi.org/10.1016/J.MICROC.2022.108261

Alotaibi, H., Hatahet, T., & Al-Jamal, W. T. (2025). Understanding the formulation parameters for engineering indocyanine green J-aggregate lipid nanocapsules and solid lipid nanoparticles as promising photothermal agents. *European Journal of Pharmaceutical Sciences*, *207*, 107034. https://doi.org/10.1016/J.EJPS.2025.107034

Ames, B. N., Lee, F. D., & Durston, W. E. (1973). An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens. *Proceedings of the National Academy*

of Sciences of the United States of America, *70*(3), 782. https://doi.org/10.1073/PNAS.70.3.782

Ates, G., Vanhaecke, T., Rogiers, V., & Rodrigues, R. M. (2017). Assaying cellular viability using the neutral red uptake assay. *Methods in Molecular Biology*, *1601*, 19–26. https://doi.org/10.1007/978-1-4939-6960-9_2,

Ayvazyan, R. G., & Jacoby, K. (2023). Kinetics and spectroscopic characterization of silica nanoparticles formed while etching quartz in hydrofluoric acid. *Materials Chemistry and Physics*, *305*, 127881. https://doi.org/10.1016/J.MATCHEMPHYS.2023.127881

Babaei, A. B., Dara, A., & Taheri, H. S. (2025). Efficacious removal of mercury metal in food industry sewerage utilizing reduced graphene oxide adsorbent composited with magnetic nanoparticles. *South African Journal of Chemical Engineering*, *52*, 97–103. https://doi.org/10.1016/J.SAJCE.2025.01.002

Bae, S. H., Yoo, S., Lee, J., Park, H. J., Kwon, S. P., Jin, H., Park, S. I., Lee, Y. S., Bang, Y. J., Roh, G., Lee, S., Youn, S. B., Kim, I. W., Oh, H. R., El-Damasy, A. K., Keum, G., Kim, H., Youn, H., Nam, J. H., & Bang, E. K. (2024). A lipid nanoparticle platform incorporating trehalose glycolipid for exceptional mRNA vaccine safety. *Bioactive Materials*, *38*, 486–498. https://doi.org/10.1016/J.BIOACTMAT.2024.05.012

Bagley, E. M., & Wages, N. A. (2025). Dose-finding designs for cell therapy cancer clinical trials evaluating drug-combinations. *Contemporary Clinical Trials*, *153*, 107894. https://doi.org/10.1016/J.CCT.2025.107894

Bajpai, S. K., Jadaun, M., & Tiwari, S. (2016). Synthesis, characterization and antimicrobial applications of zinc oxide nanoparticles loaded gum acacia/poly(SA) hydrogels. *Carbohydrate Polymers*, *153*, 60–65. https://doi.org/10.1016/J.CARBPOL.2016.07.019

Bangham, A. D., & Horne, R. W. (1964). Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. *Journal of Molecular Biology*, *8*(5), 660-IN10. https://doi.org/10.1016/S0022-2836(64)80115-7

Bangham, A. D., Standish, M. M., & Miller, N. (1965). Cation Permeability of Phospholipid Model Membranes: Effect of Narcotics. *Nature 1965 208:5017*, *208*(5017), 1295–1297. https://doi.org/10.1038/2081295a0

Bangham, A. D., Standish, M. M., & Watkins, J. C. (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology*, *13*(1), 238-IN27. https://doi.org/10.1016/S0022-2836(65)80093-6

Bansal, S., Tomer, A., Singh, A., Tyagi, N., Kushwaha, H. R., & Jain, P. (2025). In-vitro assay studies and molecular docking of

functionalized chitosan decorated vanadium pentoxide nano-agents as an antidiabetic drug. *International Journal of Biological Macromolecules*, *298*, 139986. https://doi.org/10.1016/J.IJBIOMAC.2025.139986

Barman, D., & Sarma, B. K. (2020). Thin and flexible transparent conductors with superior bendability having Al-doped ZnO layers with embedded Ag nanoparticles prepared by magnetron sputtering. *Vacuum*, *177*, 109367. https://doi.org/10.1016/J.VACUUM.2020.109367

Baskaran, A., Elumalai, M., Nagaraj, G., kasthuri, J., Vellaichamy, E., & Rajendiran, N. (2024). Mucoadhesive and drug release of cholic acid based thiomeric micelles and encapsulated silver and gold nanoparticles for anticancer studies. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *703*, 135363. https://doi.org/10.1016/J.COLSURFA.2024.135363

Baumann, N., Baumgarten, J., Kunick, C., & Bunjes, H. (2024). Prolonged release from lipid nanoemulsions by modification of drug lipophilicity. *Journal of Controlled Release*, *374*, 478–488. https://doi.org/10.1016/J.JCONREL.2024.08.021

Ben Amor, I., Hemmami, H., Grara, N., Aidat, O., Ben Amor, A., Zeghoud, S., & Bellucci, S. (2024). Chitosan: A Green Approach to Metallic Nanoparticle/Nanocomposite Synthesis and Applications. *Polymers 2024, Vol. 16, Page 2662*, *16*(18), 2662. https://doi.org/10.3390/POLYM16182662

Ben Amor, I., Hemmami, H., Laouini, S. E., Mahboub, M. S., & Barhoum, A. (2022). Sol-Gel Synthesis of ZnO Nanoparticles Using Different Chitosan Sources: Effects on Antibacterial Activity and Photocatalytic Degradation of AZO Dye. *Catalysts 2022, Vol. 12, Page 1611*, *12*(12), 1611. https://doi.org/10.3390/CATAL12121611

Benchelia, S., Messai, Y., Chetoui, A., & Mekki, D. E. (2024). Enhancing antibacterial activity through controlled ball milling: Structural, morphological and optical studies of ZnO nanoparticles. *Nano-Structures & Nano-Objects*, *39*, 101285. https://doi.org/10.1016/J.NANOSO.2024.101285

Bhardwaj, N., Kumar, A., Kaur, P., Naincy, Arora, S., Bedi, N., & Bhatia, A. (2025). Erucin-loaded chitosan coated solid lipid hybrid nanoparticles: An efficient drug delivery system for enhancing solubility and sustained release. *International Journal of Biological Macromolecules*, *308*, 142350. https://doi.org/10.1016/J.IJBIOMAC.2025.142350

Binici, B., Rattray, Z., Zinger, A., & Perrie, Y. (2025). Exploring the impact of commonly used ionizable and pegylated lipids on mRNA-LNPs: A combined in vitro and preclinical perspective. *Journal of Controlled Release*, *377*, 162–173. https://doi.org/10.1016/J.JCONREL.2024.11.010

Borisenkova, A. A., Bolshakova, O. I., Titova, A. V., Ryabokon, I. S., Markova, M. A., Lyutova, Z. B., Sedov, V. P., Varfolomeeva, E. Y.,

Bakhmetyev, V. V., Arutyunyan, A. V., Burdakov, V. S., & Sarantseva, S. V. (2024). Fullerene C60 Conjugate with Folic Acid and Polyvinylpyrrolidone for Targeted Delivery to Tumor Cells. *International Journal of Molecular Sciences*, *25*(10), 5350. https://doi.org/10.3390/IJMS25105350/S1

Bozza, A., Marengo, A., Camisassa, E., Valsania, M. C., Ugazio, E., Benetti, E., Guglielmo, S., Argenziano, M., Pisani, M., Sereno, A., Macagno, N., Accorinti, M., Quaglino, P., & Battaglia, L. (2025). Green solid lipid nanoparticles by coacervation of fatty acids: An innovative cosmetic ingredient for the delivery of anti-age compounds through the skin. *International Journal of Pharmaceutics*, *671*, 125233. https://doi.org/10.1016/J.IJPHARM.2025.125233

Broudic, K., Amberg, A., Schaefer, M., Spirkl, H. P., Bernard, M. C., & Desert, P. (2022). Nonclinical safety evaluation of a novel ionizable lipid for mRNA delivery. *Toxicology and Applied Pharmacology*, *451*, 116143. https://doi.org/10.1016/J.TAAP.2022.116143

Bruinink, A., & Luginbuehl, R. (2011). Evaluation of Biocompatibility Using In Vitro Methods: Interpretation and Limitations. *Advances in Biochemical Engineering/Biotechnology*, *126*, 117–152. https://doi.org/10.1007/10_2011_111

Castellano-Hinojosa, A., Gallardo-Altamirano, M. J., Santo Svierzoski, N. D., Pozo, C., González-López, J., & González-Martínez, A.

(2025). Anticancer drugs alter active nitrogen-cycling communities with effects on the nitrogen removal efficiency of a continuous-flow aerobic granular sludge system. *Chemosphere*, *376*, 144279. https://doi.org/10.1016/J.CHEMOSPHERE.2025.144279

Chatterjee, S., Das, A., Datta, P., Thomas, S., & Ghosal, K. (2025). Medium molecular weight chitosan and magnetite based bead as a nanocomposite for delivery of anticancer drug: Development, evaluation and biocompatibility study. *International Journal of Biological Macromolecules*, *293*, 139246. https://doi.org/10.1016/J.IJBIOMAC.2024.139246

Chen, H., Islam, W., Halabi, J. El, Li, L., & Selaru, F. M. (2025). Innovative Gastrointestinal Drug Delivery Systems: Nanoparticles, Hydrogels, and Microgrippers. *Frontiers in Bioscience - Landmark*, *30*(2), 25281. https://doi.org/10.31083/FBL25281/PDF

Chen, T., Gao, Y., Chen, X., Dong, Y., Wang, S., Huang, Q., Lin, S., Wang, J., Liao, M., & Fan, H. (2025). Self-assembling nanoparticle vaccine elicits a robust protective immune response against avian influenza H5N6 virus in chickens. *International Journal of Biological Macromolecules*, *287*, 138405. https://doi.org/10.1016/J.IJBIOMAC.2024.138405

Chen, W., Wei, Y., Chang, J., Hui, Y., Ye, J., Weng, G., Li, M., Wang, Y., & Wu, Q. (2025). Electrostimulation combined with

biodegradable electroactive oriented nanofiber polycaprolactone/gelatin/carbon nanotube to accelerate wound healing. *Materials Today Bio*, *31*, 101490. https://doi.org/10.1016/J.MTBIO.2025.101490

Chen, Z., Su, M., Xu, J., Li, J., Wangcao, G., & Qiu, L. (2024). A biocompatible glycogen based nanoparticle coating with lipid bilayer for intracellular delivery of survivin siRNA to HeLa cells. *Journal of Drug Delivery Science and Technology*, *92*, 105371. https://doi.org/10.1016/J.JDDST.2024.105371

Chen, Z., Ye, K., Wu, H., Peng, L., & Chen, Z. (2025). Thumb-sized 3D-Printed cymbal microneedle array (CyMA) for enhanced transdermal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *207*, 114629. https://doi.org/10.1016/J.EJPB.2025.114629

Chettupalli, A. K., Unnisa, A., Peddapalli, H., Jadi, R. K., Anusha, K., & Amarachinta, P. R. (2025). Development and evaluation of empagliflozin-loaded solid lipid nanoparticles: Pharmacokinetics and pharmacodynamics for oral delivery. *Intelligent Pharmacy*. https://doi.org/10.1016/J.IPHA.2024.12.004

Chiovaro, P., Girard, L., Quartararo, A., Basile, S., Bongiovì, G., Di Maio, P. A., Moscato, I., Vallone, E., & Zhou, G. (2025). Coolant contamination in gas-cooled reactors due to neutronic sputtering. *Annals of Nuclear Energy*, *213*, 111125. https://doi.org/10.1016/J.ANUCENE.2024.111125

Choi, W. H., Jo, H. R., Jeon, E. J., Youm, S. Y., Jeon, J. S., Son, Y. G., You, W. K., Koh, W. S., Lee, S. H., & Kim, S. K. (2016). Development, validation, and application of ELISA for detection of anti-HD105 antibodies in pre-clinical safety evaluation using monkeys. *Journal of Pharmaceutical and Biomedical Analysis*, *131*, 309–315. https://doi.org/10.1016/J.JPBA.2016.09.009

Comley, J. C. W., & Turner, C. H. (1990). Potential of a soluble tetrazolium/formazan assay for the evaluation of filarial viability. *International Journal for Parasitology*, *20*(2), 251–255. https://doi.org/10.1016/0020-7519(90)90107-X

Dave, R., Shah, U., Patel, A., Patel, R., & Patel, M. (2025a). Unlocking the potential of novel hybrid lipid polymer nanoparticles in redefining camptothecin delivery: Achieving controlled release, enhanced cytotoxicity, and improved pharmacokinetics for colorectal cancer therapy. *Journal of Drug Delivery Science and Technology*, *105*, 106644. https://doi.org/10.1016/J.JDDST.2025.106644

Dave, R., Shah, U., Patel, A., Patel, R., & Patel, M. (2025b). Unlocking the potential of novel hybrid lipid polymer nanoparticles in redefining camptothecin delivery: Achieving controlled release, enhanced cytotoxicity, and improved pharmacokinetics for colorectal cancer therapy. *Journal of Drug Delivery Science and Technology*, *105*, 106644. https://doi.org/10.1016/J.JDDST.2025.106644

David Rathnaraj, J., Dinesh, C., Velmurugan, R., & Adithya, A. (2022). Effect of particle size on TiO2-polystyrene nanoparticles contact angle for hydrophobicity behavior. *Materials Today: Proceedings*, *66*, 1107–1111. https://doi.org/10.1016/J.MATPR.2022.04.906

DAVIS, S. S., WASHINGTON, C., WEST, P., ILLUM, L., LIVERSIDGE, G., STERNSON, L., & KIRSH, R. (1987). Lipid Emulsions as Drug Delivery Systems. *Annals of the New York Academy of Sciences*, *507*(1), 75–88. https://doi.org/10.1111/J.1749-6632.1987.TB45793.X

De Carvalho, M., Queiroz, V., & Muehlmann, L. A. (2024). Characteristics and Preparation of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers. *Journal of Nanotheranostics 2024, Vol. 5, Pages 188-211*, *5*(4), 188–211. https://doi.org/10.3390/JNT5040012

de Sousa, A. L. N., Rebouças, L. M., Lemos, F. M. F., Ribeiro, A. C. B., Cunha, F. E. T., Pessoa, C., Sales, S. L. A., Silva, L. M. R., & Ricardo, N. M. P. S. (2025). Nanoemulsion based on Calotropis procera seed oil to delivery of betulinic acid: In vitro release kinetics, in vivo toxicity and MTT assay in PC3 cells. *Journal of Drug Delivery Science and Technology*, *106*, 106746. https://doi.org/10.1016/J.JDDST.2025.106746

Desai, N., Pande, S., Salave, S., Singh, T. R. R., & Vora, L. K. (2024). Antitoxin nanoparticles: design considerations, functional mechanisms, and applications in toxin neutralization. *Drug*

Discovery Today, *29*(8), 104060. https://doi.org/10.1016/J.DRUDIS.2024.104060

Desponds, A., Banyasz, A., Chateau, D., Tellal, A., Venier, A., Meille, S., Montagnac, G., Chevalier, J., Andraud, C., Baldeck, P. L., & Parola, S. (2021). 3D Printing and Pyrolysis of Optical ZrO2 Nanostructures by Two-Photon Lithography: Reduced Shrinkage and Crystallization Mediated by Nanoparticles Seeds. *Small*, *17*(42), 2102486. https://doi.org/10.1002/SMLL.202102486

Development & Approval Process | Drugs | FDA. (n.d.). Retrieved November 18, 2024, from https://www.fda.gov/drugs/development-approval-process-drugs

Díaz, L., Zambrano, E., Flores, M. E., Contreras, M., Crispín, J. C., Alemán, G., Bravo, C., Armenta, A., Valdés, V. J., Tovar, A., Gamba, G., Barrios-Payán, J., Bobadilla, N. A., Díaz, L., Zambrano, E., Flores, M. E., Contreras, M., Crispín, J. C., Alemán, G., … Bobadilla, N. A. (2021). Ethical Considerations in Animal Research: The Principle of 3R's. *Revista de Investigación Clínica*, *73*(4), 199–209. https://doi.org/10.24875/RIC.20000380

Domińska, K., Urbanek, K. A., Kowalska, K., Habrowska-Górczyńska, D. E., Kozieł, M. J., Ochędalski, T., & Piastowska-Ciesielska, A. W. (2024). The consequences of manipulating relaxin family peptide receptor 1 (RXFP1) level in ovarian cancer cells. *Reproductive Biology*, *24*(2), 100864. https://doi.org/10.1016/J.REPBIO.2024.100864

Dutta, B., Kumar Das, S., Temgire, M., Bellare, J., Barick, K. C., Kumar, A., & Hassan, P. A. (2024). Selective induction of apoptotic cell death in lung carcinoma cells by curcumin-loaded PEGylated lipid nanoparticles with minimal normal tissue toxicity: in vitro and in vivo toxicity evaluation by oral delivery. *Materials Advances*, *5*(10), 4504–4523. https://doi.org/10.1039/D4MA00050A

El-Baz, N., Nunn, B. M., Bates, P. J., & O'Toole, M. G. (2022). The Impact of PEGylation on Cellular Uptake and In Vivo Biodistribution of Gold Nanoparticle MRI Contrast Agents. *Bioengineering 2022, Vol. 9, Page 766*, *9*(12), 766. https://doi.org/10.3390/BIOENGINEERING9120766

Elkateb, H., Cauldbeck, H., Niezabitowska, E., Hogarth, C., Arnold, K., Rannard, S., & McDonald, T. O. (2023). High drug loading solid lipid nanoparticles, nanostructured lipid carriers and nanoemulsions for the dual drug delivery of the HIV drugs darunavir and ritonavir. *JCIS Open*, *11*, 100087. https://doi.org/10.1016/J.JCISO.2023.100087

Endla, P. (2022). Fabrication and characterization of iron (Fe) nanoparticles by Hall-Williamson, ball milling and X-ray diffraction method. *Materials Today: Proceedings*, *68*, 1571–1574. https://doi.org/10.1016/J.MATPR.2022.07.239

Enhanced Drug Delivery Using Surface-Roughened MgO@Silica Nanoparticles Loaded with Doxorubicin. (2025). *Microporous and Mesoporous Materials*, 113624. https://doi.org/10.1016/J.MICROMESO.2025.113624

Eriksson, I., Vainikka, L., Persson, H. L., & Öllinger, K. (2023). Real-Time Monitoring of Lysosomal Membrane Permeabilization Using Acridine Orange. *Methods and Protocols*, *6*(4). https://doi.org/10.3390/MPS6040072,

Espinola-Portilla, F., d'Orlyé, F., Molina González, J. A., Trapiella-Alfonso, L., Gutiérrez-Granados, S., Varenne, A., & Ramírez-García, G. (2025). Upconverting/magnetic Janus-like nanoparticles integrated into spiropyran micelle-like nanocarriers for NIR light- and pH- responsive drug delivery, photothermal therapy and biomedical imaging. *Colloids and Surfaces B: Biointerfaces*, *249*, 114501. https://doi.org/10.1016/J.COLSURFB.2025.114501

Ezhumalai, N., Nandhagopal, M., & Rajendiran, N. (2025). Synthesis of 3,6 dihydroxyflavone capped silver nanoparticles: Its antimicrobial, drug delivery and toxicity assessment. *Inorganic Chemistry Communications*, *176*, 114217. https://doi.org/10.1016/J.INOCHE.2025.114217

Ezhumalai, N., Nanthagopal, M., Kasthuri, J., & Rajendiran, N. (2024). Synthesis of N-acetylcysteine functionalized cholic acid based triarmed poly DL-Lactide and encapsulation of gold nanoparticles:

Studies on the antimicrobial activity and biocompatibility for drug delivery applications. *International Journal of Biological Macromolecules*, *279*, 135085. https://doi.org/10.1016/J.IJBIOMAC.2024.135085

Farghadin, M., Haghighi, R. D., Hosseinabadi, N., & Jafari, E. (2023). Hydrothermal synthesis of multiferroics ferrite bismuth nanoparticles with lanthanum and barium: Structural and magnetic properties investigation. *Journal of Molecular Structure*, *1286*, 135505. https://doi.org/10.1016/J.MOLSTRUC.2023.135505

Feng, S., Raimi-Abraham, B. T., & Vllasaliu, D. (2025). PEG-PLGA nanoparticles transport across in vitro intestinal epithelial models and show potential for oral delivery of antibodies in inflammatory bowel disease. *Journal of Drug Delivery Science and Technology*, *108*, 106925. https://doi.org/10.1016/J.JDDST.2025.106925

Ferreyra Maillard, A. P. V., Jimenez, L. E., Álvarez, R. M. S., Dalmasso, P. R., & Hollmann, A. (2025). Physicochemical and biological characterization and applications of silver nanoparticles obtained by green synthesis using Cichorium intybus. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *709*, 136075. https://doi.org/10.1016/J.COLSURFA.2024.136075

Gao, X., Xu, C., Yin, H., Chen, P., Wang, X., Song, Q., & Liu, J. (2020). Synthesis of nano titanium oxide with controlled oxygen content using pulsed discharge in water. *Advanced Powder Technology*, *31*(3), 986–992. https://doi.org/10.1016/J.APT.2019.12.021

Gaur, D. K., Agrahari, K., Singh, B. P., Alam, M. B., Parmar, A. S., Manohar, R., & Singh, S. (2023). Optical properties and zeta potential of polyvinyl pyrrolidone capped gold nanoparticles dispersed nematic liquid crystal mixture E7. *Optical Materials*, *145*, 114317. https://doi.org/10.1016/J.OPTMAT.2023.114317

Geetha, P., Latha, M. S., Pillai, S. S., Deepa, B., Santhosh Kumar, K., & Koshy, M. (2016). Green synthesis and characterization of alginate nanoparticles and its role as a biosorbent for Cr(VI) ions. *Journal of Molecular Structure*, *1105*, 54–60. https://doi.org/10.1016/J.MOLSTRUC.2015.10.022

Ghasemi, M., Govahi, M., & Litkohi, H. R. (2025). Green synthesis of silver nanoparticles (AgNPs) and chitosan-coated silver nanoparticles (CS-AgNPs) using Ferula gummosa Boiss. gum extract: A green nano drug for potential applications in medicine. *International Journal of Biological Macromolecules*, *291*, 138619. https://doi.org/10.1016/J.IJBIOMAC.2024.138619

Glazkova, E. A., Bakina, O. V., Rodkevich, N. G., Mosunov, A. A., Vornakova, E. A., Chzhou, V. R., & Lerner, M. I. (2022a). Copper ferrite/copper oxides (I, II) nanoparticles synthesized by electric explosion of wires for high performance photocatalytic and antibacterial applications. *Materials Science and Engineering: B*, *283*, 115845. https://doi.org/10.1016/J.MSEB.2022.115845

Glazkova, E. A., Bakina, O. V., Rodkevich, N. G., Mosunov, A. A., Vornakova, E. A., Chzhou, V. R., & Lerner, M. I. (2022b). Copper

ferrite/copper oxides (I, II) nanoparticles synthesized by electric explosion of wires for high performance photocatalytic and antibacterial applications. *Materials Science and Engineering: B*, *283*, 115845. https://doi.org/10.1016/J.MSEB.2022.115845

Gloeckner, H., Jonuleit, T., & Lemke, H. D. (2001). Monitoring of cell viability and cell growth in a hollow-fiber bioreactor by use of the dye Alamar Blue™. *Journal of Immunological Methods*, *252*(1–2), 131–138. https://doi.org/10.1016/S0022-1759(01)00347-7

Gong, L. J., Li, Y. F., Zou, H. Y., & Huang, C. Z. (2020). Resonance light scattering technique for sensitive detection of heparin using plasmonic Cu2-xSe nanoparticles. *Talanta*, *216*, 120967. https://doi.org/10.1016/J.TALANTA.2020.120967

Gopan, G., Jose, J., Khot, K. B., Bandiwadekar, A., & Deshpande N, S. (2025). Hyaluronic acid-based hesperidin nanostructured lipid carriers loaded dissolving microneedles: A localized delivery approach loaded for the management of obesity. *International Journal of Biological Macromolecules*, *305*, 140948. https://doi.org/10.1016/J.IJBIOMAC.2025.140948

Güdümcüoğlu, N., Sevim Ünlütürk, S., Çelik, İ. M., Yener, H. B., & Helvaci, Ş. Ş. (2025). Development of surface modified SiO2 nanoparticles incorporated clearcoats for automotive industry. *Surfaces and Interfaces*, *56*, 105491. https://doi.org/10.1016/J.SURFIN.2024.105491

Guo, L., Liang, H., An, D., & Yang, H. (2023). One-step hydrothermal synthesis of uniform Ag-doped SnO2 nanoparticles for highly sensitive ethanol sensing. *Physica E: Low-Dimensional Systems and Nanostructures*, *151*, 115717. https://doi.org/10.1016/J.PHYSE.2023.115717

Guo, Q., Xu, X., Lai, X., Duan, J., Yan, D., & Wang, D. (2024). Antigen/adjuvant-free liposome induces adjuvant effects for enhancing cancer immunotherapy. *Exploration*, 20230115. https://doi.org/10.1002/EXP.20230115

Halawa, E. M., Fadel, M., Al-Rabia, M. W., Behairy, A., Nouh, N. A., Abdo, M., Olga, R., Fericean, L., Atwa, A. M., El-Nablaway, M., & Abdeen, A. (2023). Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. *Frontiers in Pharmacology*, *14*, 1305294. https://doi.org/10.3389/FPHAR.2023.1305294/REFERENCE

HAMID, K. M., WAIS, M., & SAWANT, G. (2021). A REVIEW ON NANOEMULSIONS: FORMULATION, COMPOSITION, AND APPLICATIONS. *Asian Journal of Pharmaceutical and Clinical Research*, *14*, 22–28. https://doi.org/10.22159/AJPCR.2021.V14I4.40859

Hao, R. T., Chen, J., Zhao, L. H., Liu, C., Wang, O. C., Huang, G. L., Zhang, X. H., & Zhao, J. (2012). Sentinel lymph node biopsy using carbon nanoparticles for Chinese patients with papillary thyroid

microcarcinoma. *European Journal of Surgical Oncology*, *38*(8), 718–724. https://doi.org/10.1016/j.ejso.2012.02.001

Harshiny, M., Iswarya, C. N., & Matheswaran, M. (2015). Biogenic synthesis of iron nanoparticles using Amaranthus dubius leaf extract as a reducing agent. *Powder Technology*, *286*, 744–749. https://doi.org/10.1016/J.POWTEC.2015.09.021

Hawadak, J., Chaudhry, S., Pande, V., & Singh, V. (2023). Comparison of SYBR green I and lactate dehydrogenase antimalarial in vitro assay in Plasmodium falciparum field isolates. *Journal of Pharmacological and Toxicological Methods*, *124*. https://doi.org/10.1016/J.VASCN.2023.107472

Henn, J. G., Aguirre, T. A. S., Nugent, M., & Moura, D. J. (2024). Cancer nanomedicine: Recent developments in drug delivery systems and strategies to overcome eventual barriers to achieve a better outcome. *Journal of Drug Delivery Science and Technology*, *91*, 105254. https://doi.org/10.1016/J.JDDST.2023.105254

Hernández-Varela, J. D., Chanona-Pérez, J. J., Calderón Benavides, H. A., Cervantes Sodi, F., & Vicente-Flores, M. (2021). Effect of ball milling on cellulose nanoparticles structure obtained from garlic and agave waste. *Carbohydrate Polymers*, *255*, 117347. https://doi.org/10.1016/J.CARBPOL.2020.117347

Hilburg, S. L., Sokolova, A., Cagnes, M., & Pozzo, L. D. (2025). Time-resolved small-angle neutron scattering for characterization of molecular exchange in lipid nanoparticle therapeutics. *Journal of*

Colloid and Interface Science, *677*, 387–395. https://doi.org/10.1016/J.JCIS.2024.08.061

Hou, X., Zaks, T., Langer, R., & Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. *Nature Reviews Materials 2021 6:12*, *6*(12), 1078–1094. https://doi.org/10.1038/s41578-021-00358-0

Huan, K., Li, Y., Deng, D., Wang, H., Wang, D., Li, M., & Luo, L. (2022). Composite-controlled electrospinning of CuSn bimetallic nanoparticles/carbon nanofibers for electrochemical glucose sensor. *Applied Surface Science*, *573*, 151528. https://doi.org/10.1016/J.APSUSC.2021.151528

Huang, Y., Verduijn, J., Coenye, T., Liu, P., Skirtach, A. G., & Parakhonskiy, B. V. (2025). High-load, sustained-release antibacterial composite particles based on modified vaterite coated with quaternary ammonium chitosan and aldehyde hyaluronic acid for controlled drug delivery systems. *International Journal of Biological Macromolecules*, *305*, 141047. https://doi.org/10.1016/J.IJBIOMAC.2025.141047

Huzum, B., Puha, B., Necoara, R. M., Gheorghevici, S., Puha, G., Filip, A., Sirbu, P. D., & Alexa, O. (2021). Biocompatibility assessment of biomaterials used in orthopedic devices: An overview (Review). *Experimental and Therapeutic Medicine*, *22*(5), 1315. https://doi.org/10.3892/ETM.2021.10750

Iioka, S., Kanazawa, T., & Kondo, H. (2025). Design of functional peptide-assembled polymer nanoparticles with uniform dispersion

for oligonucleotide delivery to the brain and spinal cord via the nose-to-brain route. *Journal of Drug Delivery Science and Technology*, *105*, 106617. https://doi.org/10.1016/J.JDDST.2025.106617

Ishiyama, M., Miyazono, Y., Sasamoto, K., Ohkura, Y., & Ueno, K. (1997). A highly water-soluble disulfonated tetrazolium salt as a chromogenic indicator for NADH as well as cell viability. *Talanta*, *44*(7), 1299–1305. https://doi.org/10.1016/S0039-9140(97)00017-9

Jadhav, K., Jhilta, A., Singh, R., Sharma, S., Negi, S., Ahirwar, K., Shukla, R., Singh, A. K., & Verma, R. K. (2025). Trans-nasal brain delivery of anti-TB drugs by methyl-β-cyclodextrin microparticles show efficient mycobacterial clearance from central nervous system. *Journal of Controlled Release*, *378*, 671–686. https://doi.org/10.1016/J.JCONREL.2024.12.031

Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, *7*(1), 1–7. https://doi.org/10.15171/JHP.2018.01

Javed, S., Abbas, G., Shah, S., Rasul, A., Irfan, M., Saleem, A., Hosny, K. M., Bukhary, S. M., Safhi, A. Y., Sabei, F. Y., Majrashi, M. A., Alkhalidi, H. M., Alissa, M., Khan, S. M., & Hanif, M. (2023). Tobramycin-loaded nanoparticles of thiolated chitosan for ocular drug delivery: Preparation, mucoadhesion and pharmacokinetic evaluation. *Heliyon*, *9*(9), e19877.

https://doi.org/10.1016/J.HELIYON.2023.E19877

Juling, S., Niedzwiecka, A., Böhmert, L., Lichtenstein, D., Selve, S., Braeuning, A., Thünemann, A. F., Krause, E., & Lampen, A. (2017). Protein Corona Analysis of Silver Nanoparticles Links to Their Cellular Effects. *Journal of Proteome Research*, *16*(11), 4020–4034. https://doi.org/10.1021/ACS.JPROTEOME.7B00412/SUPPL_FILE/PR7B00412_SI_001.XLSX

Kelle, D., Speth, K. R., Martínez-Negro, M., Mailänder, V., Landfester, K., & Iyisan, B. (2025a). Effect of protein corona on drug release behavior of PLGA nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics*, *207*, 114611. https://doi.org/10.1016/J.EJPB.2024.114611

Kelle, D., Speth, K. R., Martínez-Negro, M., Mailänder, V., Landfester, K., & Iyisan, B. (2025b). Effect of protein corona on drug release behavior of PLGA nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics*, *207*, 114611. https://doi.org/10.1016/J.EJPB.2024.114611

Khadem Sadigh, M., Sayyar, Z., Mohammadi, M. A., & Baharlounezhad, F. (2025). Controlling the drug delivery efficiency of chitosan-based systems through silver nanoparticles and oxygen plasma. *International Journal of Biological Macromolecules*, *294*, 139407. https://doi.org/10.1016/J.IJBIOMAC.2024.139407

Khalifeh, M., Oude Egberink, R., Roverts, R., & Brock, R. (2025). Incorporation of ionizable lipids into the outer shell of lipid-coated calcium phosphate nanoparticles boosts cellular mRNA delivery. *International Journal of Pharmaceutics*, *670*, 125109. https://doi.org/10.1016/J.IJPHARM.2024.125109

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, *12*(7), 908–931. https://doi.org/10.1016/J.ARABJC.2017.05.011

Khan, Y., Sadia, H., Ali Shah, S. Z., Khan, M. N., Shah, A. A., Ullah, N., Ullah, M. F., Bibi, H., Bafakeeh, O. T., Ben Khedher, N., Eldin, S. M., Fadhl, B. M., & Khan, M. I. (2022). Classification, Synthetic, and Characterization Approaches to Nanoparticles, and Their Applications in Various Fields of Nanotechnology: A Review. *Catalysts 2022, Vol. 12, Page 1386*, *12*(11), 1386. https://doi.org/10.3390/CATAL12111386

Khashan, K. S., Sulaiman, G. M., Abdulameer, F. A., Albukhaty, S., Ibrahem, M. A., Al-Muhimeed, T., & Alobaid, A. A. (2021). Antibacterial Activity of TiO2 Nanoparticles Prepared by One-Step Laser Ablation in Liquid. *Applied Sciences 2021, Vol. 11, Page 4623*, *11*(10), 4623. https://doi.org/10.3390/APP11104623

Khattiya, A., Karaket, R., Mathaweesansurn, A., & Detsri, E. (2025). Ultrasensitive electrochemical aptasensors based on trimetallic AuPt-Ru nanoparticles decorated RGO with disposable and low-cost goldleaf electrode for aflatoxin B1 quantification in

agricultural products. *Microchemical Journal*, *208*, 112631. https://doi.org/10.1016/J.MICROC.2024.112631

Kim, S. J., & Chung, B. H. (2016). Antioxidant activity of levan coated cerium oxide nanoparticles. *Carbohydrate Polymers*, *150*, 400–407. https://doi.org/10.1016/J.CARBPOL.2016.05.021

Kimura, I., Nojima, H., & Kimura, M. (2000). Analysis of Chemical-Structure-Activity Relationships to Identify New Pro-Drugs with Unique Mechanisms of Actions in Kampo Medicines and Other Natural Products. *Studies in Natural Products Chemistry*, *24*(PART E), 875–932. https://doi.org/10.1016/S1572-5995(00)80057-5

Kondalkar, V. S., Polshettiwar, P. P., Choudhari, G. B., Pawar, A. T., & Choudhari, V. P. (2025). Evaluation of antidiabetic potential, cardio, pancreatic, nephroprotective effect, and herb-herb-drug interaction of Madhukiran formulations in T2 diabetic rats. *Phytomedicine Plus*, *5*(2), 100756. https://doi.org/10.1016/J.PHYPLU.2025.100756

Koopman, G., Reutelingsperger, C. P. M., Kuijten, G. A. M., Keehnen, R. M. J., Pals, S. T., & Van Oers, M. H. J. (1994). Annexin V for Flow Cytometric Detection of Phosphatidylserine Expression on B Cells Undergoing Apoptosis. *Blood*, *84*(5), 1415–1420. https://doi.org/10.1182/BLOOD.V84.5.1415.1415

Krasner, C. N., Campos, S. M., Young, C. L., Chadda, K. R., Lee, H., Birrer, M. J., Horowitz, N. S., Konstantinopoulos, P. A.,

D'Ascanio, A. M., Matulonis, U. A., & Penson, R. T. (2021). Sequential Phase II clinical trials evaluating CRLX101 as monotherapy and in combination with bevacizumab in recurrent ovarian cancer. *Gynecologic Oncology*, *162*(3), 661–666. https://doi.org/10.1016/J.YGYNO.2021.07.002,

Kumar, P., Mathpal, M. C., Ghosh, S., Inwati, G. K., Maze, J. R., Duvenhage, M. M., Roos, W. D., & Swart, H. C. (2022). Plasmonic Au nanoparticles embedded in glass: Study of TOF-SIMS, XPS and its enhanced antimicrobial activities. *Journal of Alloys and Compounds*, *909*, 164789. https://doi.org/10.1016/J.JALLCOM.2022.164789

Kumari, M., Kamat, S., & Jayabaskaran, C. (2022). Usnic acid induced changes in biomolecules and their association with apoptosis in squamous carcinoma (A-431) cells: A flow cytometry, FTIR and DLS spectroscopic study. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *274*, 121098. https://doi.org/10.1016/J.SAA.2022.121098

Kyriakides, T. R., Raj, A., Tseng, T. H., Xiao, H., Nguyen, R., Mohammed, F. S., Halder, S., Xu, M., Wu, M. J., Bao, S., & Sheu, W. C. (2021). Biocompatibility of nanomaterials and their immunological properties. *Biomedical Materials (Bristol, England)*, *16*(4), 10.1088/1748-605X/abe5fa. https://doi.org/10.1088/1748-605X/ABE5FA

Lan, Y., Wang, L., Lin, X., Wu, Y., & Huang, Z. (2025). Evaluation of the implementation effectiveness of accelerated drug marketing registration procedures in China: lessons from the 2016–2024 regulatory review. *Drug Discovery Today*, *30*(5), 104353. https://doi.org/10.1016/J.DRUDIS.2025.104353

Lee, B., Lee, Y., Lee, N., Kim, D., & Hyeon, T. (2025). Design of oxide nanoparticles for biomedical applications. *Nature Reviews Materials 2025*, 1–16. https://doi.org/10.1038/s41578-024-00767-x

Lee, S., & Rhee, J. S. (2025). An optimized method for estimating toxicity and cell death in the mysid model using acridine orange staining. *Molecular and Cellular Toxicology*, 1–8. https://doi.org/10.1007/S13273-025-00560-Z/FIGURES/4

Lee, W., Lee, S. W., Bae, S., Hwang, J. K., Kim, Y., Jeong, S. H., Hwang, J. S., Lee, S., Kim, D., Kang, Y., & Lee, H. S. (2025). Unraveling the effect of working pressure on the morphological, structural, optical, and compositional properties of PbI2 thin films deposited by RF magnetron sputtering. *Applied Surface Science*, *684*, 161888. https://doi.org/10.1016/J.APSUSC.2024.161888

Lee, W., Nam, J., Park, J., Lee, G., Ahn, S. hoon, Kim, K. S., Lee, J. O., Choi, C. J., & Hwang, J. Y. (2023). Single process of pulsed wire discharge for defect healing and reduction of graphene oxide. *Carbon*, *201*, 1184–1192. https://doi.org/10.1016/J.CARBON.2022.10.019

Li, C., Wang, D., & Kong, L. (2021). Application of Machine Learning Techniques in Mineral Classification for Scanning Electron Microscopy - Energy Dispersive X-Ray Spectroscopy (SEM-EDS) Images. *Journal of Petroleum Science and Engineering*, *200*, 108178. https://doi.org/10.1016/J.PETROL.2020.108178

Li, C., Yang, K., Zhang, Y., Tang, H., Yan, F., Tan, L., Xie, Q., & Yao, S. (2011). Highly biocompatible multi-walled carbon nanotube–chitosan nanoparticle hybrids as protein carriers. *Acta Biomaterialia*, *7*(8), 3070–3077. https://doi.org/10.1016/J.ACTBIO.2011.05.005

Li, M., Chang, C., Yeh, T. Y., Lee, T. Y., Lee, K. C., & Cho, E. C. (2025). Molecular characterizations of nanodiamond and related carbon-based nanomaterials in the human cell line and zebrafish models. *Diamond and Related Materials*, *152*, 111946. https://doi.org/10.1016/J.DIAMOND.2025.111946

Li, Y., Liu, F., Abdiryim, T., Xu, F., You, J., Chen, J., Yin, H., Su, M., Chen, L., Jing, X., & Liu, X. (2024). Polyimide-based triazine-cored covalent organic frameworks supported metal (Fe, Ni, Ru and Rh) nanoparticles for high-efficiency electrocatalytic hydrogen evolution. *International Journal of Hydrogen Energy*, *94*, 53–62. https://doi.org/10.1016/J.IJHYDENE.2024.11.006

Liu, J., Wang, X., Yuan, L., Zhao, Y., Zhang, Y., Wu, Y., & Liu, M. (2025). Hairpin DNA-functionalized gold nanoparticles for diagnostic imaging and overcoming doxorubicin resistance:

enhanced drug loading and cytotoxicity based on thermodynamic guidance. *Journal of Molecular Structure*, 142264. https://doi.org/10.1016/J.MOLSTRUC.2025.142264

Liu, N., & Tang, M. (2020). Toxic effects and involved molecular pathways of nanoparticles on cells and subcellular organelles. In *Journal of Applied Toxicology* (Vol. 40, Issue 1, pp. 16–36). John Wiley and Sons Ltd. https://doi.org/10.1002/jat.3817

Liu, X., Wang, C., Wang, X., Tian, C., Shen, Y., & Zhu, M. (2021). A dual-targeting Fe3O4@C/ZnO-DOX-FA nanoplatform with pH-responsive drug release and synergetic chemo-photothermal antitumor in vitro and in vivo. *Materials Science and Engineering: C*, *118*, 111455. https://doi.org/10.1016/J.MSEC.2020.111455

Liu, Y., Zhang, Y., Dong, X., Wang, J., Li, D., & Zheng, H. (2025). Controllable delivery of dual-drugs for combination therapy of chemotherapy and photodynamic therapy based on pH-responsive hyaluronic acid. *International Journal of Biological Macromolecules*, *305*, 141213. https://doi.org/10.1016/J.IJBIOMAC.2025.141213

López-García, S., Aznar-Cervantes, S. D., Pagán, A., Llena, C., Forner, L., Sanz, J. L., García-Bernal, D., Sánchez-Bautista, S., Ceballos, L., Fuentes, V., Melo, M., Rodríguez-Lozano, F. J., & Oñate-Sánchez, R. E. (2024). 3D Graphene/silk fibroin scaffolds enhance dental pulp stem cell osteo/odontogenic differentiation. *Dental Materials*, *40*(3), 431–440.

https://doi.org/10.1016/J.DENTAL.2023.12.009

Lozhkomoev, A. S., Bakina, O. V., Kazantsev, S. O., Glazkova, E. A., Rodkevich, N. G., & Lerner, M. I. (2023). Antibacterial electro-explosive Co/CoO composite nanoparticles: Synthesis, structure, magnetic and antibacterial properties. *Journal of Magnetism and Magnetic Materials*, *580*, 170892. https://doi.org/10.1016/J.JMMM.2023.170892

Lu, D., Yang, J., Wang, X., Gao, S., Li, M., & Ji, H. (2025). Effect of sputtering power on the microstructure and properties of silver films prepared by magnetron sputtering. *Materials Today Communications*, *42*, 111288. https://doi.org/10.1016/J.MTCOMM.2024.111288

Lu, H., Zha, S., Zhang, W., Wang, Q., Jiang, D., Xu, X., Zheng, X., Qiu, M., & Shan, C. (2021). A systematic review and meta-analysis of nab-paclitaxel mono-chemotherapy for metastatic breast cancer. *BMC Cancer*, *21*(1), 1–15. https://doi.org/10.1186/S12885-021-08441-Z/TABLES/3

Ma, H., Yang, Z., Xu, D., Ding, H., Liu, H., & Huang, Y. (2025). PDMS-in-water emulsions stabilized by cellulose/chitin/starch nanoparticles for fabrication of oil adsorbents: A comparison study. *Carbohydrate Polymers*, *352*, 123229. https://doi.org/10.1016/J.CARBPOL.2025.123229

Mahbubul, I. M., Saidur, R., Amalina, M. A., & Niza, M. E. (2016). Influence of ultrasonication duration on rheological properties of

nanofluid: An experimental study with alumina–water nanofluid. *International Communications in Heat and Mass Transfer*, *76*, 33–40. https://doi.org/10.1016/J.ICHEATMASSTRANSFER.2016.05.014

Malakar, C., Patowary, K., & Deka, S. (2025). Synthesis, Characterization, and Evaluation of Antimicrobial Efficacy of Sophorolipid-Coated Zinc Oxide Nanoparticles. *BioNanoScience*, *15*(1), 1–10. https://doi.org/10.1007/S12668-024-01724-2/METRICS

Mandal, N., Das, A., & Moirangthem, R. S. (2023). In-situ label-free optical biosensing with plasmonic enhanced ellipsometry using partially-embedded bimetallic Ag-Au alloy nanoparticles. *Sensors and Actuators B: Chemical*, *379*, 133164. https://doi.org/10.1016/J.SNB.2022.133164

Manzoor, S. I., Jabeen, F., Patel, R., Alam Rizvi, M. M., Imtiyaz, K., Malik, M. A., & Dar, T. A. (2025). Green synthesis of biocompatible silver nanoparticles using Trillium govanianum rhizome extract: comprehensive biological evaluation and in silico analysis. *Materials Advances*, *6*(2), 682–702. https://doi.org/10.1039/D4MA00959B

McCartan, R., Khorkova, O., Volmar, C. H., & Wahlestedt, C. (2023). Nucleic acid-based therapeutics for the treatment of central

nervous system disorders. *Frontiers in Genetics*, *14*, 1250276. https://doi.org/10.3389/FGENE.2023.1250276/FULL

McGrath, F., Qian, J., Gwynne, K., Kumah, C., Daly, D., Hrelescu, C., Zhang, X., O'Carroll, D. M., & Louise Bradley, A. (2021). Structural, optical, and electrical properties of silver gratings prepared by nanoimprint lithography of nanoparticle ink. *Applied Surface Science*, *537*, 147892. https://doi.org/10.1016/J.APSUSC.2020.147892

Mehta, M., Bui, T. A., Yang, X., Aksoy, Y., Goldys, E. M., & Deng, W. (2023). Lipid-Based Nanoparticles for Drug/Gene Delivery: An Overview of the Production Techniques and Difficulties Encountered in Their Industrial Development. *ACS Materials Au*, *3*(6), 600–619. https://doi.org/10.1021/ACSMATERIALSAU.3C00032/ASSET/IMAGES/LARGE/MG3C00032_0004.JPEG

Melzi, G., Galli, C. L., Ciliutti, P., Marabottini, C., & Marinovich, M. (2022). Lack of genotoxicity of rhubarb (rhizome) in the Ames and micronucleus in vitro tests. *Toxicology Reports*, *9*, 1574–1579. https://doi.org/10.1016/J.TOXREP.2022.07.017

Menon, D., & Fairen-Jimenez, D. (2025). Guiding the rational design of biocompatible metal-organic frameworks for drug delivery. *Matter*, *8*(3), 101958. https://doi.org/10.1016/J.MATT.2025.101958

Miao, D., Xu, J., Jiang, S., Ning, X., Liu, J., & Shang, S. (2018). Crystallization temperature investigation of Cu2ZnSnS4 by using Differential scanning calorimetry (DSC). *Ceramics International*, *44*(4), 4256–4261. https://doi.org/10.1016/J.CERAMINT.2017.12.006

Mohan, S., Vellakkat, M., Aravind, A., & Reka, U. (2020). Hydrothermal synthesis and characterization of Zinc Oxide nanoparticles of various shapes under different reaction conditions. *Nano Express*, *1*(3), 030028. https://doi.org/10.1088/2632-959X/ABC813

Mosleh, Z., Beygmohammadvand, M., Ghotbi Varzaneh, A., & Kameli, P. (2025). Influence of cobalt substitution in CoFe2O4 nanoparticles on structural, morphological, cation distribution, optical and magnetic properties. *Heliyon*, *11*(1), e41276. https://doi.org/10.1016/J.HELIYON.2024.E41276

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Motsoene, F., Abrahamse, H., & Dhilip Kumar, S. S. (2025). Lauric acid and tea tree oil-loaded solid lipid nanoparticles: Physicochemical characterisation and antibacterial activity against pathogenic bacteria. *Materials Today Communications*, *42*, 111331. https://doi.org/10.1016/J.MTCOMM.2024.111331

Muhammad, W., Liang, M., Wang, B., Xie, J., Ahmed, W., & Gao, C. (2025). NAC-Grafted ROS-Scavenging Polymer Nanoparticles for Modulation of Acute Lung Injury Microenvironment In Vivo. *Biomacromolecules*, *26*(1), 528–540. https://doi.org/10.1021/ACS.BIOMAC.4C01290

Munusamy, M. A., Bharathi, M., Hirad, A. H., Alarfaj, A. A., Hussein-Al-Ali, S. H., Sampath, S., & Kudumba, A. (2025). An Escin-loaded Glutaraldehyde-Albumin nanoparticle system for enhancing anticancer activity on lung cancer A549 cells. *Results in Chemistry*, *13*, 102021. https://doi.org/10.1016/J.RECHEM.2025.102021

Nguyen, H. M., Alexander, K. E., Collinge, M., Hickey, J. C., Lanz, T. A., Li, J., Sheehan, M. J., Newman, L. C., & Thorn, M. (2024). mRNA-LNPs induce immune activation and cytokine release in human whole blood assays across diverse health conditions. *Molecular Therapy*. https://doi.org/10.1016/J.YMTHE.2024.12.019

Nie, H., Huang, R., Jiang, G., Li, W., Yang, L., Zhang, M., Qian, M., Guo, W., Ye, T., & Huang, R. (2024). Modulating active targeting nanoparticle design according to tumor progressions. *Acta Pharmaceutica Sinica B*. https://doi.org/10.1016/J.APSB.2024.12.016

Nishimori, H., Kondoh, M., Isoda, K., Tsunoda, S. ichi, Tsutsumi, Y., & Yagi, K. (2009). Histological analysis of 70-nm silica particles-

induced chronic toxicity in mice. *European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V*, *72*(3), 626–629. https://doi.org/10.1016/J.EJPB.2009.03.007

Ochoa-Sánchez, C., Rodríguez-León, E., Iñiguez-Palomares, R., & Rodríguez-Beas, C. (2024). Brief Comparison of the Efficacy of Cationic and Anionic Liposomes as Nonviral Delivery Systems. *ACS Omega*, *9*(47), 46664–46678. https://doi.org/10.1021/ACSOMEGA.4C06714

Ortiz-Perez, A., van Tilborg, D., van der Meel, R., Grisoni, F., & Albertazzi, L. (2024). Machine learning-guided high throughput nanoparticle design. *Digital Discovery*, *3*(7), 1280–1291. https://doi.org/10.1039/D4DD00104D

Ostios-Garcia, L., Pérez, D. M., Castelo, B., Herradón, N. H., Zamora, P., Feliu, J., & Espinosa, E. (2024). Classification of anticancer drugs: an update with FDA- and EMA-approved drugs. *Cancer and Metastasis Reviews 2024 43:4*, *43*(4), 1561–1571. https://doi.org/10.1007/S10555-024-10188-5

Osuntokun, J., & Ajibade, P. A. (2016). Morphology and thermal studies of zinc sulfide and cadmium sulfide nanoparticles in polyvinyl alcohol matrix. *Physica B: Condensed Matter*, *C*(496), 106–112. https://doi.org/10.1016/J.PHYSB.2016.05.024

Pandey, P., Jakhmola, V., Saha, S., & Gaurav, A. (2025). Recent Developments in Diabetes Management: Exploring Receptors,

Pathways, and Compounds. *BIO Integration*, *6*(1). https://doi.org/10.15212/BIOI-2024-0137

Patel, A. K., Sumathi, Y., Singhania, R. R., Chen, C. W., Michaud, P., & Dong, C. Di. (2025). Innovative graphene quantum dots applications for enhancing lutein biosynthesis in microalgae biorefineries. *Chemical Engineering Journal*, *505*, 159744. https://doi.org/10.1016/J.CEJ.2025.159744

Patel, M., Mishra, S., Verma, R., & Shikha, D. (2022). Synthesis of ZnO and CuO nanoparticles via Sol gel method and its characterization by using various technique. *Discover Materials* , *2*(1), 1–11. https://doi.org/10.1007/S43939-022-00022-6/FIGURES/10

Peng, L., An, Y., Xiang, H., Pan, X., Wang, Y., Yang, Q., Cao, X., Wang, Z. L., & Zhang, L. (2024). Liquid metal complexing Fe3O4 nanoparticles enable rapid polymerization of magnetically conductive hydrogels for various flexible electronics. *Nano Energy*, *130*, 110058. https://doi.org/10.1016/J.NANOEN.2024.110058

Pensado-López, A., Fernández-Rey, J., Reimunde, P., Crecente-Campo, J., Sánchez, L., & Torres Andón, F. (2021). Zebrafish Models for the Safety and Therapeutic Testing of Nanoparticles with a Focus on Macrophages. *Nanomaterials 2021, Vol. 11, Page 1784*, *11*(7), 1784. https://doi.org/10.3390/NANO11071784

Phull, A. R., Ali, A., Dhong, K. R., Zia, M., Mahajan, P. G., & Park, H. J. (2021). Synthesis, characterization, anticancer activity

assessment and apoptosis signaling of fucoidan mediated copper oxide nanoparticles. *Arabian Journal of Chemistry*, *14*(8), 103250. https://doi.org/10.1016/J.ARABJC.2021.103250

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J. L., Pérez Marc, G., Moreira, E. D., Zerbini, C., Bailey, R., Swanson, K. A., Roychoudhury, S., Koury, K., Li, P., Kalina, W. V., Cooper, D., Frenck, R. W., Hammitt, L. L., … Gruber, W. C. (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *New England Journal of Medicine*, *383*(27), 2603–2615. https://doi.org/10.1056/NEJMOA2034577/SUPPL_FILE/NEJMOA2034577_DATA-SHARING.PDF

Prabakaran, A., Rakshit, D., Patel, I., Susanna, K. J., Mishra, A., Radhakrishnanand, P., Sarma, P., & Alexander, A. (2025). Chitosan-coated nanostructured lipid carriers for intranasal delivery of sinapic acid in Aβ1–42 induced C57BL/6 mice for Alzheimer's disease treatment. *International Journal of Biological Macromolecules*, *305*, 141136. https://doi.org/10.1016/J.IJBIOMAC.2025.141136

Prajapat, M., Gholap, A. D., Shinde, S., Padhiyar, D., Butani, S., Shah, S., & Rajput, A. (2025). 3D printing technology in microneedles: An emerging era in transdermal drug delivery. *Hybrid Advances*, *10*, 100447. https://doi.org/10.1016/J.HYBADV.2025.100447

Pushpamalini, T., Keerthana, M., Sangavi, R., Nagaraj, A., & Kamaraj, P. (2021). Comparative analysis of green synthesis of TiO2

nanoparticles using four different leaf extract. *Materials Today: Proceedings*, *40*, S180–S184. https://doi.org/10.1016/J.MATPR.2020.08.438

Pusuwan, P., & Siripinyanond, A. (2024). Observing zinc oxide nanoparticles suspension stability in various media by using single particle inductively coupled plasma mass spectrometry (SP-ICP-MS). *Microchemical Journal*, *196*, 109705. https://doi.org/10.1016/J.MICROC.2023.109705

Puvvada, N., Shaik, M. A. S., Samanta, D., Shaw, M., Mondal, I., Basu, R., Bhattacharya, A., & Pathak, A. (2024). Biocompatible fluorescent carbon nanoparticles as nanocarriers for targeted delivery of tamoxifen for regression of Breast carcinoma. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *321*, 124721. https://doi.org/10.1016/J.SAA.2024.124721

Qayyum, H., Hussain, S., Ahmed, W., Al-Ahmadi, A. N., & Abdel-Aty, A. H. (2025). Effect of surface charge on laser-produced silver nanoparticles for dye reduction and surface-enhanced Raman spectroscopy. *Journal of Physics and Chemistry of Solids*, *196*, 112408. https://doi.org/10.1016/J.JPCS.2024.112408

Rajendrachari, S., Taslimi, P., Karaoglanli, A. C., Uzun, O., Alp, E., & Jayaprakash, G. K. (2021). Photocatalytic degradation of Rhodamine B (RhB) dye in waste water and enzymatic inhibition study using cauliflower shaped ZnO nanoparticles synthesized by

a novel One-pot green synthesis method. *Arabian Journal of Chemistry*, *14*(6), 103180. https://doi.org/10.1016/J.ARABJC.2021.103180

Rami, J. M., Patel, C. D., Patel, C. M., & Patel, M. V. (2021). Thermogravimetric analysis (TGA) of some synthesized metal oxide nanoparticles. *Materials Today: Proceedings*, *43*, 655–659. https://doi.org/10.1016/J.MATPR.2020.12.554

Ramos-Medina, M. J., Echeverría-Garcés, G., Kyriakidis, N. C., León Cáceres, Á., Ortiz-Prado, E., Bautista, J., Pérez-Meza, Á. A., Abad-Sojos, A., Nieto-Jaramillo, K., Espinoza-Ferrao, S., Ocaña-Paredes, B., & López-Cortés, A. (2024). CardiOmics signatures reveal therapeutically actionable targets and drugs for cardiovascular diseases. *Heliyon*, *10*(1), e23682. https://doi.org/10.1016/J.HELIYON.2023.E23682

Rampado, R., Crotti, S., Caliceti, P., Pucciarelli, S., & Agostini, M. (2020). Recent Advances in Understanding the Protein Corona of Nanoparticles and in the Formulation of "Stealthy" Nanomaterials. *Frontiers in Bioengineering and Biotechnology*, *8*, 166. https://doi.org/10.3389/FBIOE.2020.00166

Rane, R., Prabhune, A., Kumar, D., Kumar, A., Sewlikar, M., Suryawanshi, M., Satpute, B., Gawade, B., & Sakat, S. (2024). Levothyroxine synthesis impurities are neither mutagenic nor genotoxic: Insilico, Ames test and micronucleus test studies. *Mutation Research - Genetic Toxicology and Environmental*

Mutagenesis, *898*, 503796. https://doi.org/10.1016/J.MRGENTOX.2024.503796

Rashid, T. M., Nayef, U. M., Jabir, M. S., & Mutlak, F. A. H. (2021). Synthesis and characterization of Au:ZnO (core:shell) nanoparticles via laser ablation. *Optik*, *244*, 167569. https://doi.org/10.1016/J.IJLEO.2021.167569

Ray, T. R., Lettiere, B., De Rutte, J., & Pennathur, S. (2015). Quantitative characterization of the colloidal stability of metallic nanoparticles using UV-vis absorbance spectroscopy. *Langmuir*, *31*(12), 3577–3586. https://doi.org/10.1021/LA504511J/SUPPL_FILE/LA504511J_SI_002.AVI

Recordati, C., De Maglie, M., Bianchessi, S., Argentiere, S., Cella, C., Mattiello, S., Cubadda, F., Aureli, F., D'Amato, M., Raggi, A., Lenardi, C., Milani, P., & Scanziani, E. (2016). Tissue distribution and acute toxicity of silver after single intravenous administration in mice: nano-specific and size-dependent effects. *Particle and Fibre Toxicology*, *13*(1), 12. https://doi.org/10.1186/S12989-016-0124-X

Rodrigues, R. M., Stinckens, M., Ates, G., & Vanhaecke, T. (2023). Neutral Red Uptake Assay to Assess Cytotoxicity In Vitro. *Methods in Molecular Biology*, *2644*, 237–245. https://doi.org/10.1007/978-1-0716-3052-5_15

Ruiz, E., Orozco, V. H., Hoyos, L. M., & Giraldo, L. F. (2022a). Study of sonication parameters on PLA nanoparticles preparation by simple emulsion-evaporation solvent technique. *European Polymer Journal*, *173*, 111307. https://doi.org/10.1016/J.EURPOLYMJ.2022.111307

Ruiz, E., Orozco, V. H., Hoyos, L. M., & Giraldo, L. F. (2022b). Study of sonication parameters on PLA nanoparticles preparation by simple emulsion-evaporation solvent technique. *European Polymer Journal*, *173*, 111307. https://doi.org/10.1016/J.EURPOLYMJ.2022.111307

Sahin, U., Muik, A., Derhovanessian, E., Vogler, I., Kranz, L. M., Vormehr, M., Baum, A., Pascal, K., Quandt, J., Maurus, D., Brachtendorf, S., Lörks, V., Sikorski, J., Hilker, R., Becker, D., Eller, A. K., Grützner, J., Boesler, C., Rosenbaum, C., … Türeci, Ö. (2020). COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature*, *586*(7830), 594–599. https://doi.org/10.1038/S41586-020-2814-7;TECHMETA

Sallustio, V., Rossi, M., Marto, J., Coelho, T., Chinnici, F., Mandrone, M., Chiocchio, I., Cappadone, C., Luppi, B., Bigucci, F., Abruzzo, A., & Cerchiara, T. (2024). Green extraction of Rosa canina L. and Prunus spinosa L. by NaDES and their encapsulation in chitosan nanoparticles for cosmetic industry. *Industrial Crops and Products*, *218*, 119042. https://doi.org/10.1016/J.INDCROP.2024.119042

Santhanes, D., Zhang, H., Wilkins, A., John Aitken, R., Gannon, A. L., & Liang, M. (2024). Engineering pH-sensitive dissolution of lipid-polymer nanoparticles by Eudragit integration impacts plasmid DNA (pDNA) transfection. *European Journal of Pharmaceutics and Biopharmaceutics*, *199*. https://doi.org/10.1016/j.ejpb.2024.114299

Sasaki, T., & Ohno, T. (1994). Cytotoxicity tests on eye drop preparations by LDH release assay in human cultured cell lines. *Toxicology in Vitro : An International Journal Published in Association with BIBRA*, *8*(5), 1113–1119. https://doi.org/10.1016/0887-2333(94)90252-6

Satofuka, H., Suzuki, H., Tanaka, T., Ubukata, R., Hirose, M., Yamamoto, H., Kaneko, Y., Fujisawa, S., Li, G., Kaneko, M. K., & Kato, Y. (2025). A novel anti-mouse CCR7 monoclonal antibody, C7Mab-7, demonstrates high sensitivity in flow cytometry, western blot, and immunohistochemistry. *Biochemistry and Biophysics Reports*, *41*, 101948. https://doi.org/10.1016/J.BBREP.2025.101948

Schlattmann, D., Weber, B., Wyszynski, L., Schönhoff, M., & Haas, H. (2024). Molecular localization and exchange kinetics in pharmaceutical liposome and mRNA lipoplex nanoparticle products determined by small angle X-ray scattering and pulsed field gradient NMR diffusion measurements. *European Journal of Pharmaceutics and Biopharmaceutics*, *201*, 114380. https://doi.org/10.1016/J.EJPB.2024.114380

Schütz, C. A., Juillerat-Jeanneret, L., Mueller, H., Lynch, I., & Riediker, M. (2013). Therapeutic nanoparticles in clinics and under clinical evaluation. *Nanomedicine-Uk*, *8*(3), 449–467. https://doi.org/10.2217/nnm.13.8

Sempel, J. D., Kaariainen, M.-L., Colleran, T. A., Lifschitz, A. M., & George, S. M. (2024). Ultrathin ZrO2 thickness control on TiO2/ZrO2 core/shell nanoparticles using ZrO2 atomic layer deposition and etching. *Journal of Vacuum Science & Technology A*, *42*(5). https://doi.org/10.1116/6.0003781/3309065

Senapati, S., Tripathi, K., Awad, K., & Rahimipour, S. (2024). Multifunctional Liposomes Targeting Amyloid-β Oligomers for Early Diagnosis and Therapy of Alzheimer's Disease. *Small*, *20*(31), 2311670. https://doi.org/10.1002/SMLL.202311670

Shulga, V. I. (2024). Simulation of ion beam sputtering with OKSANA and SRIM: A comparative study. *Vacuum*, *230*, 113644. https://doi.org/10.1016/J.VACUUM.2024.113644

Siebert, D. A., Caon, N. B., Alberton, M. D., Vitali, L., Parize, A. L., & Micke, G. A. (2023). Immobilized acetylcholinesterase in magnetic nanoparticles for in-line inhibition studies using a capillary electrophoresis system. *Analytica Chimica Acta*, *1275*, 341566. https://doi.org/10.1016/J.ACA.2023.341566

Singh, A., Ahmed, A., Sharma, A., Sharma, C., Paul, S., Khosla, A., Gupta, V., & Arya, S. (2021). Promising photocatalytic degradation of methyl orange dye via sol-gel synthesized Ag–

CdS@Pr-TiO2 core/shell nanoparticles. *Physica B: Condensed Matter*, *616*, 413121. https://doi.org/10.1016/J.PHYSB.2021.413121

Singh, K., Singhal, S., Pahwa, S., Sethi, V. A., Sharma, S., Singh, P., Kale, R. D., Ali, S. W., & Sagadevan, S. (2024). Nanomedicine and drug delivery: A comprehensive review of applications and challenges. *Nano-Structures & Nano-Objects*, *40*, 101403. https://doi.org/10.1016/J.NANOSO.2024.101403

Solar-assisted synthesis of silver nanoparticles from Amphilophium paniculatum (L.) Kunth: Unlocking multi-therapeutic potential for lung cancer, diabetes and drug resistant infections through In vitro studies and In silico antidiabetic evaluations. (2025). *Microbial Pathogenesis*, *205*, 107647. https://doi.org/10.1016/J.MICPATH.2025.107647

Soni, V., Jain, P., Haider, T., Pandey, V., & Tekade, R. K. (2018). Documentation Protocol in Product Development Including Clinical Records. *Dosage Form Design Parameters*, *2*, 403–440. https://doi.org/10.1016/B978-0-12-814421-3.00012-9

Soyalp, S., Eksin, E., & Erdem, A. (2025). Chitosan and carbon nanotube modified DNA biosensor for determination of drug-DNA interaction. *Journal of Pharmaceutical and Biomedical Analysis Open*, *5*, 100050. https://doi.org/10.1016/J.JPBAO.2025.100050

Steć, A., Jońca, J., Waleron, K., Waleron, M., Płoska, A., Kalinowski, L., Wielgomas, B., & Dziomba, S. (2022). Quality Control of Bacterial Extracellular Vesicles with Total Protein Content Assay, Nanoparticles Tracking Analysis, and Capillary Electrophoresis. *International Journal of Molecular Sciences*, *23*(8), 4347. https://doi.org/10.3390/IJMS23084347/S1

Stenspil, S. G., & Laursen, B. W. (2024). Photophysics of fluorescent nanoparticles based on organic dyes – challenges and design principles. *Chemical Science*, *15*(23), 8625–8638. https://doi.org/10.1039/D4SC01352B

Sun, D., & Lu, Z. R. (2023). Structure and Function of Cationic and Ionizable Lipids for Nucleic Acid Delivery. *Pharmaceutical Research*, *40*(1), 27. https://doi.org/10.1007/S11095-022-03460-2

Syed, B., Prasad, NagendraM. N., & Satisha, S. (2016). Endogenic mediated synthesis of gold nanoparticles bearing bactericidal activity. *Journal of Microscopy and Ultrastructure*, *4*(3), 162–166. https://doi.org/10.1016/J.JMAU.2016.01.004

Taheri, Z., Mirjalili, M. H., Shahsavarani, H., Ghassempour, A., & Rahmandoust, M. (2025). Single-step synthesized carbon quantum dots from Centella asiatica hairy roots: Photoluminescent, biocompatibility, antibacterial and anticancer activity. *Industrial Crops and Products*, *229*, 120999. https://doi.org/10.1016/J.INDCROP.2025.120999

Talaei, F., Farzad, F., & Raygan, Z. (2025). A study on fullerene as a polymer-assisted antiepileptic drug delivery system: A non-covalent interaction. *Journal of Molecular Liquids*, *419*, 126765. https://doi.org/10.1016/J.MOLLIQ.2024.126765

Tamargo, J., Agewall, S., Borghi, C., Ceconi, C., Cerbai, E., Dan, G. A., Ferdinandy, P., Grove, E. L., Rocca, B., Magavern, E., Sulzgruber, P., Semb, A. G., Sossalla, S., Niessner, A., Kaski, J. C., & Dobrev, D. (2024). New pharmacological agents and novel cardiovascular pharmacotherapy strategies in 2023. *European Heart Journal — Cardiovascular Pharmacotherapy*, *10*(3), 219. https://doi.org/10.1093/EHJCVP/PVAE013

Tan, Y. F., Hii, L. W., Lim, W. M., Cheong, S. K., Leong, C. O., Yee, M. S. L., & Mai, C. W. (2024). Polyethylene glycol-phospholipid functionalized single-walled carbon nanotubes for enhanced siRNA systemic delivery. *Scientific Reports 2024 14:1*, *14*(1), 1–14. https://doi.org/10.1038/s41598-024-80646-1

Targhazeh, N., Maleki, M., Alemi, F., & Yousefi, B. (2023). Enhanced drug loading capacity of graphene oxide nanoparticles by polyglycerol hyper branching and increasing the sensitivity of osteosarcoma cancer cells to doxorubicin. *Journal of Drug Delivery Science and Technology*, *88*, 104871. https://doi.org/10.1016/J.JDDST.2023.104871

Tatovic, D., Mcateer, M. A., Barry, J., Barrientos, A., Rodríguez Terradillos, K., Perera, I., Kochba, E., Levin, Y., Dul, M.,

Coulman, S. A., Birchall, J. C., Von Ruhland, C., Howell, A., Stenson, R., Alhadj Ali, M., Luzio, S. D., Dunseath, G., Cheung, W. Y., Holland, G., … Ludvigsson, J. (2022). Safety of the use of gold nanoparticles conjugated with proinsulin peptide and administered by hollow microneedles as an immunotherapy in type 1 diabetes. *Immunotherapy Advances*, *2*(1), ltac002. https://doi.org/10.1093/IMMADV/LTAC002

The Drug Development Process | FDA. (n.d.). Retrieved November 18, 2024, from https://www.fda.gov/patients/learn-about-drug-and-device-approvals/drug-development-process

Tian, X., & Yuan, Y. (2024). Impacts of polyethylene glycol (PEG) dispersity on protein adsorption, pharmacokinetics, and biodistribution of PEGylated gold nanoparticles. *RSC Advances*, *14*(29), 20757–20764. https://doi.org/10.1039/D4RA03153A

Tian, X., Zhang, Y., & Ai, H. W. (2024). PEGylated ATP-Independent Luciferins for Noninvasive High-Sensitivity High-Speed Bioluminescence Imaging. *ACS Chemical Biology*, *20*, 128–136. https://doi.org/10.1021/ACSCHEMBIO.4C00601/SUPPL_FILE/CB4C00601_SI_002.MP4

Tian, Y., Sun, M., Song, R., Yang, Z., & Zhang, H. (2025). DNA dendrimer-based nanocarriers for targeted Co-delivery and controlled release of multiple chemotherapeutic drugs. *RSC Advances*, *15*(4), 2981–2987. https://doi.org/10.1039/D4RA07839J

Tomar, D., & Jeevanandam, P. (2020). Synthesis of cobalt ferrite nanoparticles with different morphologies via thermal decomposition approach and studies on their magnetic properties. *Journal of Alloys and Compounds*, *843*, 155815. https://doi.org/10.1016/J.JALLCOM.2020.155815

Tomar, D., & Jeevanandam, P. (2022). Synthesis of ZnFe2O4 nanoparticles with different morphologies via thermal decomposition approach and studies on their magnetic properties. *Journal of Magnetism and Magnetic Materials*, *564*, 170033. https://doi.org/10.1016/J.JMMM.2022.170033

Tyszczuk-Rotko, K., Sadok, I., & Barczak, M. (2016). Thiol-functionalized polysiloxanes modified by lead nanoparticles: Synthesis, characterization and application for determination of trace concentrations of mercury(II). *Microporous and Mesoporous Materials*, *C*(230), 109–117. https://doi.org/10.1016/J.MICROMESO.2016.04.043

Uzoeto, H. O., Nkiru, A., Aisida, S. O., & Ezema, F. I. (2024). Enhanced microstructural properties and biocompatibility of Zinc oxide nanoparticles by biogenic synthesis protocol for antibacterial applications. *Hybrid Advances*, *7*, 100332. https://doi.org/10.1016/J.HYBADV.2024.100332

Valenzuela Villela, K. S., Betancourt Galindo, R., Espinosa Neira, R., Espinosa Solís, V., & García-Casillas, P. E. (2024). Stability of starch-folic acid/ polyethylene glycol particles for gastrointestinal

drug delivery. *Food Hydrocolloids*, *157*, 110318. https://doi.org/10.1016/J.FOODHYD.2024.110318

Vankudoth, S., Dharavath, S., Veera, S., Maduru, N., Chada, R., Chirumamilla, P., Gopu, C., & Taduri, S. (2022). Green synthesis, characterization, photoluminescence and biological studies of silver nanoparticles from the leaf extract of Muntingia calabura. *Biochemical and Biophysical Research Communications*, *630*, 143–150. https://doi.org/10.1016/J.BBRC.2022.09.054

Vroman, L., Adams, A., Fischer, G., & Munoz, P. (1980). Interaction of High Molecular Weight Kininogen, Factor XII, and Fibrinogen in Plasma at Interfaces. *Blood*, *55*(1), 156–159. https://doi.org/10.1182/BLOOD.V55.1.156.156

Walter, D. L., Bian, Y., Hu, H., Hamid, F. A., Rostamizadeh, K., Vigliaturo, J. R., DeHority, R., Ehrich, M., Runyon, S., Pravetoni, M., & Zhang, C. (2025). The immunological and pharmacokinetic evaluation of Lipid-PLGA hybrid nanoparticle-based oxycodone vaccines. *Biomaterials*, *313*, 122758. https://doi.org/10.1016/J.BIOMATERIALS.2024.122758

Wang, H., Brown, P. C., Chow, E. C. Y., Ewart, L., Ferguson, S. S., Fitzpatrick, S., Freedman, B. S., Guo, G. L., Hedrich, W., Heyward, S., Hickman, J., Isoherranen, N., Li, A. P., Liu, Q., Mumenthaler, S. M., Polli, J., Proctor, W. R., Ribeiro, A., Wang, J. Y., … Huang, S. M. (2021). 3D cell culture models: Drug pharmacokinetics, safety assessment, and regulatory

consideration. *Clinical and Translational Science*, *14*(5), 1659–1680. https://doi.org/10.1111/CTS.13066

Wang, X., Fang, X., Liu, X., Lyu, Y., Ma, L., & Fu, J. (2024). Spike nanoparticles: From design to biomedical applications. *Next Materials*, *2*, 100080. https://doi.org/10.1016/J.NXMATE.2023.100080

Wang, X., Liu, G., Pu, X., Ren, T., Zhang, F., Shen, M. J., Zhu, Y., Kros, A., & Yang, J. (2025). Combating cisplatin-resistant lung cancer using a coiled-coil lipopeptides modified membrane fused drug delivery system. *Journal of Controlled Release*, *379*, 45–58. https://doi.org/10.1016/J.JCONREL.2025.01.004

Wilczewska, A. Z., Niemirowicz, K., & Markiewicz, K. H. (2012). *Review Nanoparticles as drug delivery systems*. https://doi.org/10.1016/s1734-1140(12)70901-5.

Wilson, B. K., & Prud'homme, R. K. (2021). Nanoparticle size distribution quantification from transmission electron microscopy (TEM) of ruthenium tetroxide stained polymeric nanoparticles. *Journal of Colloid and Interface Science*, *604*, 208–220. https://doi.org/10.1016/J.JCIS.2021.04.081

Wu, S., Hatahet, T., Bona, B. L., Lodigiani, G., Zhang, M., Bombelli, F. B., & Al-Jamal, W. T. (2025). Incorporating Span 80 surfactant into lipid nanocapsules improves their biocompatibility and cellular uptake in B16F10 melanoma cells. *International Journal*

of Pharmaceutics, *672*, 125358. https://doi.org/10.1016/J.IJPHARM.2025.125358

Xi, C., Tian, C., Yu, L., Xiong, Y., Tang, Z., & Chen, Z. (2025). Resonance light scattering combined with miniaturized Thermal-Assisted Purge-and-Trap device for screening of hydrochloride drugs. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *329*, 125605. https://doi.org/10.1016/J.SAA.2024.125605

Xiao, H., Meng, X., Songtao Li, Li, Z., Fang, S., Wang, Y., Li, J., Tang, J., & Ma, L. (2024). Combined drug anti-deep vein thrombosis therapy based on platelet membrane biomimetic targeting nanotechnology. *Biomaterials*, *311*, 122670. https://doi.org/10.1016/J.BIOMATERIALS.2024.122670

Xue, W., Wei, X., Xiang, Z., Zhang, X., Tu, W., He, Y., Chen, S., & Gao, D. (2025). Simplified biomimetic peptide-based vehicle for enhanced tumor penetration and rapid enzyme-induced drug release. *Journal of Colloid and Interface Science*, *684*, 75–86. https://doi.org/10.1016/J.JCIS.2024.12.237

Yadav, S., Pathak, J., Majhi, P., Kaushik, S., Shukla, A. K., Kaur, R., Kumar, R., & Singh, P. (2025). Lac-based-biosynthesis of zinc–copper mixed metal oxide nanoparticles and evaluation of their antifungal activity against A.alternata and F. oxysporum. *Materials Chemistry and Physics*, *330*, 130152. https://doi.org/10.1016/J.MATCHEMPHYS.2024.130152

Yadav, U. P., Yaseen, M., Singh, S., Babu, M. A., Bhat, M. A., Kumar, R., Tyagi, Y., Ullah, I., & Huang, Y. (2025). Design and development of new substituted pyrimidine hybrids with imidazole and triazole: Exploring utility as an anticancer agent via human topoisomerase-II and tubulin inhibition. *Bioorganic Chemistry*, *158*, 108334. https://doi.org/10.1016/J.BIOORG.2025.108334

Yang, K., Zeng, Y., Wu, X., Li, J., & Guo, J. (2024). Strategies for developing self-assembled nanoparticle vaccines against SARS-CoV-2 infection. *Frontiers in Immunology*, *15*. https://doi.org/10.3389/FIMMU.2024.1392898

Yen, T. Y. C., Abbasi, A. Z., He, C., Lip, H. Y., Park, E., Amini, M. A., Adissu, H. A., Foltz, W., Rauth, A. M., Henderson, J., & Wu, X. Y. (2024). Biocompatible and bioactivable terpolymer-lipid-MnO2 Nanoparticle-based MRI contrast agent for improving tumor detection and delineation. *Materials Today Bio*, *25*, 100954. https://doi.org/10.1016/J.MTBIO.2024.100954

Yokaichiya, F., Schmidt, C., Storsberg, J., Kumpugdee Vollrath, M., de Araujo, D. R., Kent, B., Clemens, D., Wingert, F., & Franco, M. K. K. D. (2018). Effects of doxorubicin on the structural and morphological characterization of solid lipid nanoparticles (SLN) using small angle neutron scattering (SANS) and small angle X-ray scattering (SAXS). *Physica B: Condensed Matter*, *551*, 191–196. https://doi.org/10.1016/J.PHYSB.2017.12.036

Younas, M., Rizwan, M., Zubair, M., Inam, A., & Ali, S. (2021). Biological synthesis, characterization of three metal-based nanoparticles and their anticancer activities against hepatocellular carcinoma HepG2 cells. *Ecotoxicology and Environmental Safety*, *223*, 112575. https://doi.org/10.1016/J.ECOENV.2021.112575

Yusuf, A., Almotairy, A. R. Z., Henidi, H., Alshehri, O. Y., & Aldughaim, M. S. (2023). Nanoparticles as Drug Delivery Systems: A Review of the Implication of Nanoparticles' Physicochemical Properties on Responses in Biological Systems. *Polymers 2023, Vol. 15, Page 1596*, *15*(7), 1596. https://doi.org/10.3390/POLYM15071596

Zaman, M. S., Khosravieh, Z. F., Ahssan, M., Salehiamin, M., Ghoraishizadeh, S., Darvishnia, F., Rahmani, E., & Esmaeili, J. (2024). Fabrication of a conduit for future peripheral nerve regeneration using decellularized plant tissue modified with polyaniline/graphene oxide nanosheet. *Materials Today Communications*, *39*, 109204. https://doi.org/10.1016/J.MTCOMM.2024.109204

Zeng, J., Xuan, Y., & Li, Q. (2023). Direct solar-thermal scalable-decomposition of methanol flowing through a nanoparticle-packed bed reactor under outdoor environment. *Energy*, *280*, 128187. https://doi.org/10.1016/J.ENERGY.2023.128187

Zhang, C., Han, D. J., Lee, D. H., Lee, H. H., Lee, J. K., & Lee, B. Y. (2024). Comparison of atomic force microscopy and dynamic

light scattering on the size characterization of surfactant-modified WC/Co nanoparticles dispersed in aqueous media. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *685*, 133117. https://doi.org/10.1016/J.COLSURFA.2023.133117

Zhang, J., Pan, T., Lee, J., Dekel, B., Huang, J., Wu, X., Zhang, J., Pan, T., Lee, J., Goldberg, S., King, S. A., Tang, E., Hu, Y., & Chen, L. (2025). Enabling tumor-specific drug delivery by targeting the Warburg effect of cancer. *Cell Reports Medicine*, *6*(1), 101920. https://doi.org/10.1016/J.XCRM.2024.101920

Zhang, L., Zhang, Y., Wu, B., & Deng, H. (2023). A study of thermal etching of GaN by atmospheric argon inductively coupled plasma. *Thermochimica Acta*, *724*, 179491. https://doi.org/10.1016/J.TCA.2023.179491

Zhang, S., Guan, L., Jiang, Y., Zhao, F., Zhou, X., & Liu, Z. (2022). Resolving structure dimension for SiO2@C Core-shell nanoparticles by three-step modeling of ellipsometry. *Materials Science and Engineering: B*, *286*, 116040. https://doi.org/10.1016/J.MSEB.2022.116040

Zhang, W., Wang, S., Xing, Y., Luo, X., Wang, R., & Yu, F. (2025). Bioorthogonal SERS-bioluminescence dual-modal imaging for real-time tracking of triple-negative breast cancer metastasis. *Acta Biomaterialia*. https://doi.org/10.1016/J.ACTBIO.2025.03.019

Zhang, X., Zhang, L., Liu, Q., Li, H., Li, S., Liu, Z., Tang, F., & Feng, H. (2024). Macrophage membrane-coated Eucommia ulmoides

polysaccharides-loaded PLGA nanoparticles as an effective antigen-targeted delivery system. *Applied Materials Today*, *38*, 102173. https://doi.org/10.1016/J.APMT.2024.102173

Zhang, Y., Watson, S., Ramaswamy, Y., & Singh, G. (2024). Intravitreal therapeutic nanoparticles for age-related macular degeneration: Design principles, progress and opportunities. *Advances in Colloid and Interface Science*, *329*, 103200. https://doi.org/10.1016/J.CIS.2024.103200

Zhang, Z., & Yan, Y. (2025). Effect of loaded carbon-based nanoparticles on the evaporation dynamics of sessile droplets. *International Journal of Thermal Sciences*, *209*, 109549. https://doi.org/10.1016/J.IJTHERMALSCI.2024.109549

Zhao, L., Liu, C., Wang, T., Sun, L., Wu, F., & Yu, D. (2025). Combined multispectral analysis and molecular docking to research the interaction of soybean isolate protein with different kinds of phospholipid liposomes and its effect on liposome properties. *Food Chemistry*, *474*, 143160. https://doi.org/10.1016/J.FOODCHEM.2025.143160

Zhao, X., Yuan, L., Gong, Z., Li, M., Yuan, Y., & Geng, J. (2025). New drugs approved by the NMPA in 2024: Synthesis and clinical applications. *European Journal of Medicinal Chemistry*, *291*, 117643. https://doi.org/10.1016/J.EJMECH.2025.117643

Zheng, T., O'Neill, C., Marshall, J. F., Iskratsch, T., & Palma, M. (2024). Selective placement of functionalised DNA origami via

thermal scanning probe lithography patterning. *Materials Advances*, *5*(23), 9376–9382. https://doi.org/10.1039/D4MA00828F

Zheng, X., Guo, P., Zhang, Y., Xu, J., Sun, J., & Lei, Y. (2022). An ultra sensitive and rapid SERS detection method based on vortex aggregation enhancement effect for anti-infective drug residues detection in water. *Analytica Chimica Acta*, *1235*, 340539. https://doi.org/10.1016/J.ACA.2022.340539

Zia, A. W., Anestopoulos, I., Bowen, L., Panayiotidis, M. I., & Birkett, M. (2024). Precision depth-controlled isolated silver nanoparticle-doped diamond-like carbon coatings with enhanced ion release, biocompatibility, and mechanical performance. *Surface and Coatings Technology*, *493*, 131281. https://doi.org/10.1016/J.SURFCOAT.2024.131281

Zoe, L. H., David, S. R., & Rajabalaya, R. (2023). Chitosan nanoparticle toxicity: A comprehensive literature review of in vivo and in vitro assessments for medical applications. *Toxicology Reports*, *11*, 83–106. https://doi.org/10.1016/J.TOXREP.2023.06.012

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